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# QUANTIFYING LONG TERM PATTERNS OF FEMALE ALTERNATIVE REPRODUCTIVE TACTICS IN WOOD DUCKS (AIX SPONSA)

A dissertation submitted in partial satisfaction
of the requirements for the degree of

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ECOLOGY AND EVOLUTIONARY BIOLOGY

by

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# TABLE OF CONTENTS

# TABLE OF TABLES

## TABLE OF FIGURES

## ABSTRACT

### Quantifying Long Term Patterns of Female Alternative Reproductive Tactics in Wood Ducks (*Aix sponsa*)

by

Caroline M. Thow

All organisms must allocate limited resources to reproduction and survival, producing life history trade-offs. One such trade-off is maternal care, which balances a female's current reproduction against her future survival. Alternative reproductive tactics have evolved as a way of maximizing lifetime reproductive success by balancing the costs of parental care against the benefits that care provides to current offspring. Conspecific brood parasitism (CBP) is an alternative reproductive tactic where females lay eggs in the nest of a conspecific, providing no further care to those offspring. Some parasitic females may have their own nest in addition to laying parasitically. This results in three possible seasonal reproductive options: nesting and parasitizing, nesting without parasitizing, and parasitizing without nesting. While CBP is taxonomically widespread, it occurs at remarkably high rates in waterfowl. Detecting CBP is challenging, and historically researchers have relied on behavioral and morphological evidence to identify it. Recently, genetic methods of maternity assignment have allowed researchers to detect parasitism directly. However, genetic methods have not been examined under conditions common in waterfowl, where parasitic females may be highly related to their hosts and the candidate parent pool is often incomplete. Additionally, while females can flexibly transition between CBP

reproductive options between years, the reproductive success of CBP reproductive options are often quantified by single-season estimates and are rarely considered in the context of the entire lifespan of the females that engage in this behavior.

My dissertation focuses on testing accuracy of both genetic and non-genetic methods of detecting CBP and quantifying long-term patterns of CBP in the wood duck (*Aix sponsa*) in California. In Chapter 1, I assessed the accuracy of detecting CBP using genetic assignments with simulated wood duck populations. Genetic methods of maternity assignment in the context of CBP in wood ducks rarely make assignment errors given a diverse set of microsatellite markers and a largely complete sample of candidate mothers, but the risk of false exclusion and misassignment does increase with related females in the candidate parent pool. In Chapter 2, I compared field-based genetic and non-genetic estimates of CBP. Non-genetic methods produced underestimates of CBP as compared to genetic estimates. However, in combination with genetic estimates of CBP, non-genetic methods uncovered CBP patterns in wood ducks that genetic methods alone would not have revealed. In Chapter 3, I used genetic assignments to quantify the frequency of CBP in wood ducks at the population level over five years, calculated single-season reproductive success of individual females' reproductive options, and determined long-term patterns of CBP behavior of individual and their individual lifetime fitness estimates. The frequency of non-parasitic nesting females declined as density of breeding females and nest sites increased. Females that nested and parasitized had the highest reproductive success by single season measurements, while parasites that did not nest had the lowest

reproductive success. Nesting parasitism contributed the most offspring to lifetime reproductive success but parasitism alone or nesting without parasitism could result in large fitness gains. Females nested more frequently as they aged, with or without parasitism, and continued to do so until they exited the population. Collectively, the results of my thesis suggest that genetic methods of maternity assignment are robust in CBP waterfowl, and female wood ducks flexibly transition into nesting reproductive options as they age, which ultimately results in higher reproductive success over the course of their lifetimes. My research is one of the first studies to assess lifetime reproductive success in a CBP system and highlights the importance of taking a long-term perspective in studies of alternative reproductive options to fully understand the costs and benefits of engaging in these behaviors.

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They say it takes a village to raise a child, and I am here to tell you it takes far more people to help a graduate student get her PhD. I would not have made it through this process without the love, support, and humor of so many amazing people, both inside and outside of academia, who have lifted me up during this unbelievably challenging and rewarding process. I would not have had this opportunity at all without my advisor, Bruce Lyon, agreeing to take me on after meeting me exactly once. All I remember from that meeting was scrambling to cover up when I implied some birds might have infrared vision (none do as of this writing, I am sad to report), and Bruce laughing and agreeing that yes, that would be cool if birds could see in infrared. He has been kindly prodding me to expand my horizons in more realistic directions ever since, and his keen perspective and insight into not only my research, but into all manner of scientific inquiries is inspiring and motivating. Thank you, Bruce, for your guidance, humor, and support over the years.

Unbeknownst to me at the time, my graduate career started when I was 19 years old, when I met John Eadie for the first time. I had taken an ornithology class and enjoyed the lab so much I thought I would try continuing with a good thing and take a class on gamebird and waterfowl management. I stepped into the classroom and was almost immediately hooked. John's class introduced me to the wild and wonderful world of wood ducks and cemented my passion for field work and research. I am so thankful that, a literal decade (and then some) later, John is still on my team. His support from before I even applied to graduate school, through

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always believed I would find my way there. Sure enough, through a combination of my experience working and my desire to pursue research as a career, I decided to apply for PhD programs. My dad was an incredibly smart and inventive man, and I am proud to be his daughter as well as a part of his educational legacy; without his experience as a professor at UC Berkeley in the Music department, I would have had no idea what I was signing up for as a graduate student. My mother, Peggy Thow, is equally inspirational and supportive; I always know I have a soft place to land with her. Her generosity, kindness, and unfailing love throughout this process kept me afloat during some of the darkest times of my life and career. I hope that I have and will always make you proud, Mom, since it's the only way I know how to thank you for being such a fantastic mother (unlike some of the ducks I studied). My sister Diana is soon-to-be the next Dr. Thow in the family, and always strives to connect the dots; she pointed out that my thesis was in fact feminist, like hers, because of the focus on female reproductive options, which have been largely overlooked historically. She and I have spent many hours commiserating over the hardest aspects of graduate school and I am so happy we both made it. She also produced my adorable nephew, August, along the way, who is just as excited to talk about birds as I am. Thank you both for being such an incredibly warm and supportive family, now and always. I promise to never stop learning.

# INTRODUCTION

## BROADER CONTEXT

Life history trade-offs are fundamental to understanding the evolution and maintenance of behavioral variation within populations. A prime example of a life-history based tradeoff is the allocation of maternal care, where the mother must strike a balance between her investment in her current offspring and anticipated future reproduction (Trivers 1972; Winkler & Walters 1983). Diverse alternative reproductive tactics (ARTs) have evolved within and across species as a way of maximizing lifetime reproductive success by balancing the costs of maternal care against the benefits that care provides to current offspring.

Conspecific brood parasitism (CBP) is an example of a female ART, where a female lays eggs in the nest of a conspecific host female and provides no further care for the offspring, completely relying on the other female to successfully raise her offspring. Taxonomically, CBP is widespread, and while it has been observed in birds (Yom-Tov 1980, 2001), fish (Wisenden 1999), and insects (Zink 2000), it is particularly prevalent in waterfowl (Lyon & Eadie 2008). In waterfowl species, parasitic females may or may not have their own nest in addition to the eggs they lay parasitically (Sorenson 1991; Lyon 1993; Lyon & Eadie 2008), adding complexity to the fitness ramifications of this behavior. Non-nesting females who only reproduce through parasitism are likely making the best of a bad job (Lyon & Eadie 2018). However, the fitness payoffs for females that both nest and lay parasitically is not as

well understood, because it is unclear why nesting females would opt to lay eggs outside of their own nest.

Explanations for CBP in birds have evolved since the behavior first attracted the attention of evolutionary biologists (Yom-Tov 1980; Lyon & Eadie 2008). While CBP was initially considered an infrequent mistake made by confused or ill females, it has since been recognized as a widespread phenomenon occurring at rates that were high enough to dismiss the hypothesis that the behavior was an occasional fluke (Yom-Tov 1980, 2001). Early studies of CBP focused on identifying factors associated with increased parasitic behavior, such as density of breeding females, nest site availability, and nest loss (Yom-Tov 1980; Bellrose & Holm 1994). A diverse array of adaptive hypotheses arose from the findings of these early studies, suggesting that the evolution and maintenance of CBP could be linked to ecological and/or social restraints and constraints. Lyon and Eadie (2008) synthesized these adaptive hypotheses under a life history framework based on Sorenson's (1991) reproductive decision model, which describes CBP as an array of reproductive options that females can adopt based on their internal or external conditions to optimize their seasonal, and ultimately lifetime, reproductive success. Nesting and parasitizing is the highest investment reproductive option with the highest probability of success, followed by nesting without parasitizing, and lastly parasitism without nesting (which is higher investment than abstaining from breeding for a season). Under this model, reproductive options are stops along an investment continuum; females will adopt the

option that could provide the highest fitness gain given their current ability to invest in reproduction.

However, the hypothesis that CBP is a non-adaptive consequence of other social and ecological factors influencing females has not been conclusively ruled out; Semel and Sherman (2001) argue that CBP is a non-adaptive consequence of competition over limited nest sites. Thus, decades after the inquiries into CBP began, the mystery of why and when some females choose to parasitize has only deepened. The answer lies in understanding what fitness benefits females ultimately gain or lose by engaging in, or abstaining from, parasitism.

A vital step in investigating evolution and maintenance of CBP is obtaining accurate estimates of reproductive success, which are expressed in the currency of fitness costs and benefits in CBP systems. Historically, accurate measurements of individual female reproductive success has been difficult because parasitic offspring associate with their host females, not their biological mothers, and distinguishing between offspring of the same species is challenging (Andersson 1984). In the past, researchers detected CBP based on variation in egg appearance, abnormally large clutch sizes, exceptionally high egg accumulation rates, and actual observations of the act of parasitism (Eadie *et al.* 2010). More recently, radio frequency identification (RFID) tagging technology has allowed researchers to record extensive behavioral observations of individual breeding females in non-CBP breeding systems (Bonter & Bridge 2011). While RFID tagging has not been used in any published research on

CBP systems as of this writing, it is ideal for providing nest visitation data, and could provide key insights into parasitic female behavior.

The advent of genetic parentage assignment has given researchers another powerful tool to identify otherwise cryptic parasitic offspring and link them to their biological mothers. However, given that many studies of CBP do not completely sample all reproductive individuals and that parasitic individuals are the most likely to go undetected, it is unclear how accurately genetic methods detect incidence of parasitism within populations (Jones *et al.* 2010). Moreover, in waterfowl species, natal philopatry results in highly related females breeding in close proximity, which may make candidate mothers difficult to differentiate due to shared rare alleles among related individuals (Double *et al.* 1997; Araki & Blouin 2005). To rigorously assess our ability to accurately quantify CBP, we need to analyze genetic assignments using simulated populations of known pedigree and compare genetic to non-genetic lines of evidence of CBP in wild populations.

Accurate estimates of individual reproductive success are also critical for putting CBP reproductive options into a life history context. Many species that display CBP are iteroparous, and, therefore, adopting a reproductive option in one year may have longer term consequences that are only apparent later in the individual's lifetime (Lyon & Eadie 2018). However, most studies of CBP examine reproductive options and their correlates and consequences only within a single season. The extent to which individual females flexibly transition among reproductive options across years is unknown for most CBP species; thus, single season estimates

of reproductive success may not be predictive of overall lifetime reproductive success. Furthermore, due to life history trade-offs, highly successful single-season reproductive options may result in shorter lifespans overall or reduced reproductive success in future seasons (Taborsky 2008). Long-term studies investigating reproductive choices and their consequences over multiple seasons are required to understand the life history context of CBP and factors contributing to the evolution and maintenance of the behavior.

## DISSERTATION OVERVIEW

The goal of my dissertation is to rigorously test the accuracy of the methods used to detect CBP and quantify the long-term population- and individual-level patterns of CBP for female wood ducks (*Aix sponsa*). Wood ducks are an ideal study species to examine both the most robust methods to detect CBP and the long-term patterns and consequences of the behavior for individuals, because it is well established that wood ducks frequently engage in CBP reproductive options. Moreover, they have high natal site fidelity when they mature to breeding age and nest readily in artificial nest boxes, making it easy to track an individual's reproductive choices over multiple seasons. In Chapter 1, I used simulated wood duck populations to investigate the accuracy of genetic maternity assignment methods under two conditions common to CBP systems: 1) missing parental genotypes and 2) relatives in the candidate parent pool. In Chapter 2, I compared the methods of traditional ecological maternal assignment, RFID, and genetic maternal assignment to

detect CBP at the population level and to assign an individual to a particular reproductive option within a single season in wild wood duck populations. In Chapter 3, I applied the findings from my previous two chapters to quantify the frequency of CBP in wood ducks, the reproductive effort and success of reproductive options as determined from a single season of observation versus multiple seasons of observation, and explore how individual females transition between reproductive options over their lifetime. Collectively, my dissertation provides an in-depth examination of the methods routinely used to detect CBP and other ARTs and implements a long term life history perspective of CBP that is rarely applied to female ARTs, providing a critical guide for the design and implementation of future studies into ART systems.

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# CHAPTER 1: Are you my mommy or my auntie? Assigning parentage in populations with kin structure and incomplete parent sampling

## 1.1. INTRODUCTION

In studies of natural populations, identifying parents of offspring from a candidate parent pool is a critical step for estimating the reproductive effort and success of individuals, which is essential for understanding the evolution of reproductive strategies. Alternative reproductive tactics such as conspecific brood parasitism and extra-pair paternity make estimating reproductive effort and assigning parentage difficult because the social parent of an offspring may not be its genetic parent (Griffith *et al.* 2002; Walling *et al.* 2010; Lyon & Eadie 2018). To detect alternative reproductive tactics and strategies (ARTS), researchers have employed both observational and genetic methods. The advent of inexpensive genetic techniques in particular has allowed researchers to identify otherwise cryptic parents and elucidate breeding dynamics that were incompletely understood; molecular markers such as microsatellites and SNPs can both detect mismatches between offspring and their social parents and identify the true parents of an offspring from a pool of possible candidate parents (Petrie & Møller 1991; Arnold & Owens 2002; Avise *et al.* 2002).

Conspecific brood parasitism (CBP) is one such breeding tactic that is difficult to detect without using genetic techniques. In CBP, a breeding female lays at least one egg in the nest of another female of the same species without providing any further care for that offspring. This form of parasitism is widespread in birds and has

now been reported in over 200 species (Yom-Tov 2001). Historically, CBP has often been under-detected or even undetected (Yom-Tov 2001), since it is difficult to comprehensively detect parasitism with behavioral observations alone, and in many species it is impossible to correctly identify parasitic eggs based on visual cues (Yom-Tov 1980; Macwhirter 1989; Eadie *et al.* 2010). Depending on the species, a parasitic female in a given year may have her own nest in addition to laying parasitically, or the parasite may forgo nesting altogether, so parasites that do not have their own nests usually escape detection unless they are caught in the act of parasitism. A clear understanding of which females lay parasitically, and why, has developed slowly due to these limitations. The development of molecular markers has increased the frequency at which CBP is detected (Yom-Tov 2001), allows researchers to determine which females in the population lay the parasitic eggs, and has provided the context of parasitic laying and trade-offs with normal nesting (Lyon & Eadie 2008).

Although molecular techniques are powerful tools to elucidate reproductive dynamics with CBP, molecular parentage assignment programs are potentially susceptible to genotyping errors (Kalinowski *et al.* 2007; Jones *et al.* 2010) and they make assumptions that may not apply to every breeding system (Jones & Ardren 2003; Jones *et al.* 2010). Errors resulting from allelic dropout or null alleles lead to allelic mismatches which result in under- or overestimates of CBP, depending on the parentage assignment criteria used (Lemons *et al.* 2014). If the assumptions of perfect knowledge of the proportion of parents missing and relatedness between individuals

in the candidate parent pool (Jones *et al.* 2010) are not met, programs may falsely exclude parents when the population contains relatives or when candidate parent sampling is incomplete, biasing assignments towards relatives or sampled individuals and creating patterns unrepresentative of breeding system dynamics (Araki & Blouin 2005; Jones *et al.* 2010).

It is well established that the number of molecular markers may also influence the accuracy of genetic parentage assignment programs (Jones *et al.* 2010; Lemons *et al.* 2014). Although developing and using many genetic markers for parentage assignment is costly, using too few markers may compromise the assignment ability and reliability of parentage assignment programs. When candidate parents are related to one another, more markers provide the programs with greater power to distinguish between similar genotypes, and therefore increase the probability of correct assignments (Jones & Ardren 2003; Jones *et al.* 2010; Lemons *et al.* 2014).

Assignment programs can make two types of assignment error: 1) incorrectly leave offspring unassigned (falsely excluding the true mother) or 2) incorrectly assign offspring (falsely excluding the true mother and assigning her progeny to another female). While lack of assignment and incorrect assignment can both lead to inaccurate measures of reproductive success and rates of CBP, the way they do so is different (Table 1.1). Incorrectly leaving offspring unassigned results in a loss of information, which leads to falsely reduced measures of reproductive success in the candidate parent pool and muted patterns of parasitism. Incorrectly assigning offspring underestimates the reproductive effort for the true mother of the

misassigned offspring and overestimates the reproductive effort for the falsely assigned mother. Incorrectly assigned offspring may result in muted or misleading patterns of parasitism.

In mating systems with ARTS or CBP, the consequences of these assignment errors become more nuanced when we consider them in the context of the reproductive tactic (i.e. parasitic status) of the mother of the incorrectly assigned or unassigned offspring (Table 1.1). For example, when studies consider all unassigned offspring as parasitic (Anderholm *et al.* 2009; Tiedemann *et al.* 2011; Hario *et al.* 2012; Lemons *et al.* 2014) and assignment power is low (eg. studies with a low number of markers), there is risk that many of these unassigned 'parasites' are actually non-parasitic offspring of the female in the nest they are found in. Conversely, incorrect assignment of a truly parasitic offspring to a non-parasitic female would underestimate the frequency of CBP and the reproductive effort of parasite tactics (either non-nesting or nesting). In addition to contributing to inaccurate estimates of parasitism, incorrectly assigned parasitic offspring could potentially result in false patterns of host-parasite interactions. For example, if a program incorrectly assigned a nesting female's own offspring to a close relative, it would create apparent kin-directed parasitism when in fact there is none. Recent studies suggest that CBP may be kin-directed and cooperative rather than parasitic (Nielsen *et al.* 2006; Tiedemann *et al.* 2011; Jaatinen *et al.* 2011), but to verify this we first must test the accuracy of parentage assignment programs.

Waterfowl present a unique opportunity to evaluate the accuracy of genetic methods to assign parentage for the following reasons: CBP is disproportionally prevalent in waterfowl compared to other taxa, females are philopatric and may parasitize relatives, parasites may not nest in a given year and thus evade sampling, and male sampling is infrequent because males are often not involved in nesting (Yom-Tov 1980; Andersson 1984; Eadie *et al.* 1988; Eadie & Lyon 2011). The combination of these factors may interact in ways that reduce the accuracy of genetic parentage assignment. Female natal philopatry can result in candidate mothers being related, whose shared alleles may make it difficult for programs to distinguish between potential mothers (Double *et al.* 1997; Olsen *et al.* 2001; Jaatinen *et al.* 2011) and could result in incorrect assignment of offspring to a relative of the true mother (Jones *et al.* 2010). Compounding the issue of related candidate females is the fact that parasitic females may or may not have a nest of their own, yet many researchers are typically only able to sample nesting females because they can be captured and/or sampled at their nests (Nielsen *et al.* 2006; Jaatinen *et al.* 2009; Lemons & Sedinger 2011). This could potentially bias assignments towards those individuals present in the candidate parent pool provided to the assignment software (Nielsen *et al.* 2001; Araki & Blouin 2005). Finally, males are often absent or difficult to capture and sample in waterfowl breeding studies (Tiedemann *et al.* 2011; Jaatinen *et al.* 2011). The absence of genetic information provided by one parent (the males in CBP) may complicate the process of assigning progeny to the other parent

(the females in CBP), particularly if candidate parents are related to one another (Double *et al.* 1997).

In waterfowl species, recent studies have focused on intriguing questions on the evolution of CBP and the role of kinship in CBP. However, the intersection of female philopatry, incomplete female sampling, and lack of male sampling in these systems may pose special challenges to genetic assignment. To understand the evolutionary underpinnings of CBP in waterfowl fully, we must determine if the parentage assignment methods we use are producing results that reflect actual patterns in natural populations. Previous empirical studies outside of CBP have assessed the reliability of parentage assignment programs using a variety of approaches including: examining the effect of allele drop out and stuttering on assignment accuracy in natural populations (Araki & Blouin 2005; Berger-Wolf *et al.* 2007), comparing the results from assignment programs to ecological data to verify the accuracy of assignment programs (Walling *et al.* 2010; Guerier *et al.* 2012; Sánchez-Tójar *et al.* 2015), and comparing the accuracy of parentage assignment when analyzing a typical number of loci against a large number of loci (Walling *et al.* 2010; Karaket & Poompuang 2012). However, few studies have addressed the combined effects of incomplete parent sampling and kinship on genetic assignment accuracy (but see Double *et al.* 1997). One effective method to explore the intersection of incomplete sampling and kinship is through the use of simulations. Jones et al. (2010) emphasized the value of simulations in establishing accuracy of

genetic parentage assignment, and the need for further studies to employ this approach to produce guidelines for best practices in parentage assignment.

In this study, we used simulated populations to examine the accuracy and reliability of parentage assignment under conditions frequently observed in CBP. We created populations with completely known pedigrees based on observed allele frequency distributions from our study populations of wood ducks (*Aix sponsa*), a cavity-nesting species in which CBP is common (Semel & Sherman 2001; Nielsen *et al.* 2006; Odell & Eadie 2010). Simulated populations allow us to assess the accuracy parentage assignments using data where parentage was perfectly known (because we simulated the broods) and then compare assignment outputs to the true known parents of offspring. Our simulations included mixtures of related and unrelated females, as well as a mixture of parasitic and non-parasitic offspring. With these simulations, we then investigated which characteristics of the candidate parent pool were most essential for accurate genetic parentage assignment by conducting three runs with different candidate parent pool characteristics: 1) completeness of the candidate parent pool sampling for both sexes, which we investigated by removing male genotypes from the candidate parent pool, 2) completeness of sampling the sex of interest, which we addressed by removing a subset of candidate mother genotypes from the candidate parent pool, and 3) relatedness between candidate parents, which we addressed by constructing kin structure into our simulated populations. We also contrasted the accuracy of the two most commonly used parentage assignment

software programs, CERVUS and COLONY, and further explored how the number of markers influences the accuracy of assignments

## 1.2. METHODS

### 1.2.1. Study System and Sample Collection

Wood ducks are cavity nesting waterfowl that readily nest in nest boxes. While females produce a clutch of 10 to 15 eggs, previous studies indicate parasitized clutches contain between 16 to 22 eggs on average (Bellrose & Holm 1994), and they can have as many as 58 eggs in our study populations (Odell & Eadie 2010). Females display natal philopatry and thus may nest near and/or parasitize nesting relatives. Previous studies indicate that wood ducks are likely polygamous or seasonally monogamous, but the true nature of the mating system has yet to be conclusively determined (Bellrose & Holm 1994; Baldassarre 2014). After nest site selection and mating, male wood ducks do not provide parental care to their offspring.

To obtain the genotypes used as the basis of our simulated wood duck populations, we sampled wild wood duck populations in Yolo County, CA. From 2012 to 2015, we monitored 237 wood duck nest boxes at four sites: Conaway Ranch in Woodland, CA, Putah Creek and Russell Ranch in Davis, CA, and Roosevelt Ranch in Zamora, CA. We caught nesting females between the first and third week of incubation to band them and take a blood sample. We made additional efforts during the breeding season to band and blood sample non-nesting females in nest traps. We also deployed bait traps, which are designed to capture foraging ducks away from the

nest, after the breeding season to capture previously unbanded female ducks. We were unable to thoroughly sample male wood ducks in our populations due to their reclusive nature during the breeding season, so actual male genotypes were not included in this study. Instead, male genotypes were simulated using allele frequencies from the females in all populations combined (see below).

We collected blood samples (roughly 0.2 ml) via medial metatarsal venipuncture using a 22-gauge needle. We then either applied the sample to DNA-preserving filter paper (Adventec Nobuto blood filter strips) or collected the blood with a capillary tube and deposited it in a 0.5 ml sample of Queen's lysis buffer (Seutin *et al.* 1991). We sent unprocessed blood and samples of previously extracted DNA (extracted using either Qiagen DNEasy spin column kits or the Whitehead lab protocol, Appendix A1.1) to the UC Davis Veterinary Genetics Lab (VGL) where they were genotyped using 18 microsatellite loci developed for wood ducks and other closely related taxa (Odell 2008) (Appendix A1.1). The VGL estimated the mistyping rate to be 0.05 or less, and 98% of the loci were typed.

### 1.2.2. Simulated Populations

We investigated how accurately genetic programs can assign offspring under several potentially challenging contexts: when paternal genotypes are missing, related females are in the population, or the female sample is incomplete. We explored this by presenting the programs with breeding populations of wood ducks, each with a subset of non-nesting parasitic females that vary in their relatedness to nesting hosts.

We simulated wood duck populations of known pedigree, relatedness, and nesting status to produce offspring that could then be assigned to their parents under the various challenging conditions. We created two simulated populations of wood ducks based on empirical allele frequencies from two wild populations.

To obtain each simulated population, we needed to generate three generations: each female and male in the first generation (G1) was created from actual allele frequencies sampled from a wild wood duck population, a second generation (G2) of females of known relatedness and pedigree was produced from a simulated mating of the first generation, and a third generation (G3) of offspring was created from a simulated mating of the second-generation females with additional males from the first generation.

To generate G1 simulated female genotypes we compiled two sets of field sampled wood duck genotypes, one from each of two of our study sites: Conaway Ranch females (n=52, collected between 2012 and 2015) and Roosevelt Ranch females (n = 70, collected in 2014 and 2015). We used M-L Relate to determine the allele frequencies of each sample of females, and with these frequencies generated independent sets of 200 simulated female genotypes with COLONY. To construct simulated male duck genotypes, we selected 10 female wild duck genotypes at random from each of our four field sites and determined the allele frequencies of this sample. We then used these allele frequencies to construct 136 simulated male genotypes in COLONY, which were paired with Conaway Ranch simulated females. This method of simulating genotypes reflects the known population genetic structure

of natural wood duck populations, as the natally-philopatric females contribute genes to the same local population they hatched from and as the dispersing sex males contribute genes from outside populations. We repeated the process with a different sample of females from three of the four sites to create a second group of 136 simulated male genotypes to pair with Roosevelt Ranch simulated females. For the fourth site, Putah Creek, we only had genotypes for 10 females total; we used these genotypes in generating both sets of simulated males.

For each of the two population simulations, we randomly chose 64 simulated males to pair with 56 G1 females to produce 72 G2 simulated female offspring. These numbers were chosen to produce the desired number of full and half sib pairs of females (Figure 1.1). To produce each G2 female genotype, we randomly selected one allele from each G1 parent for each locus. Of the 72 G2 females, 16 were full siblings, 16 were maternal half siblings, and 40 were not related to another female in the population (Figure 1.1). Full sibling pairs were generated by having 8 sets of G1 parents produce two offspring. The half siblings were generated by having eight G1 females have one offspring each with two mates (hence the need for an additional 8 extra males compared to females for the G1 individuals).

We then assigned G2 females to one of two reproductive tactics, resulting in 48 nesting females and 24 parasitic females. Sixteen of the nesting females had relatives that were brood parasites (Figure 1.1); the remaining 32 nesting females were not related to any other female in the population. The nesting females only reproduced through nesting and the parasitic females only reproduced parasitically. In

the field, identification of specific parasitic females depends on whether a genetic sample is obtained from the female. When parasites also have their own nests, researchers should be able to obtain samples from the parasites because birds are typically captured at their nests, and therefore are included as candidate parents. Parasites that do not have their own nest are more likely to go unsampled, and therefore are potentially at higher risk of having their offspring assigned incorrectly to another female as their genotypes are not included in the candidate parent pool. We restricted our simulations to parasitism by females without their own nests because we felt this presents the most challenging case for parentage assignments; the lack of samples from parasitic females could most affect the outcome of maternity analyses.

The primary goal for each simulation was to assign the maternity of the ducklings in the third-generation to females in the second-generation. To create the third-generation ducklings, we paired each of the 72 second generation females with a unique mate from the remaining 72 males that had not been used as sires for the second-generation females to produce 10 ducklings per pair (720 ducklings in total). We constructed each duckling genotype by randomly selecting one allele from each parent for each locus.

### 1.2.3. Maternity Analyses

We used two of the most frequently used parentage assignment programs, COLONY and CERVUS, to obtain maternity assignments for each set of simulated offspring. Both programs use maximum likelihood approaches to assign offspring to

their parent(s), but they differ in a few key respects in their approach. CERVUS takes a pairwise maximum likelihood approach to assign offspring to their parents, using a three-step process. First, the program runs a pairwise parentage analysis on a simulated population it creates from the allele frequencies of the input genotypes from a population of interest. Second, from this simulation CERVUS generates a statistic called the critical LOD (likelihood of the odds) score that determines the level of confidence the program has in its assignments. The critical LOD score serves as a cut-off point above which CERVUS can assign a parent at a designated level of confidence in runs of parentage analysis on the focal population. For example, the critical LOD score for a 95% confidence level is determined as the LOD score value above which 19 of 20 parents selected by the simulation as the most likely parents are the actual parents of the offspring. CERVUS allows the user to assign a strict and relaxed level of confidence, which are set by default at 95% and 80% confidence level thresholds respectively. While the authors of CERVUS recommend only accepting assignments made at the 95% or greater confidence level for satisfactory assignments, some researchers have used confidence levels as low as 80% as a cut-off for assignment (Table 1.2). Any assignment made at a less than 80% confidence level indicates that the program cannot find a suitable parent in the candidate parent pool. In the final step of its parentage assignment process, CERVUS uses the critical LOD scores generated by its simulation to assign parents to offspring in the focal population: the candidate parent with the highest LOD score for an offspring is selected and a confidence level for the assignment is determined based on the where it

falls in comparison to the critical LOD scores for the pre-designated confidence levels.

In contrast, COLONY takes a pedigree level approach to assign parentage by determining the familial relationships best supported by the genetic evidence available. COLONY uses an annealing algorithm to search for the best maximum likelihood pedigree configuration amongst thousands of possibilities to assign paternity, maternity, and sibships (both full and half) in the population of interest. The user can specify details such as mating system, information about relatedness between known candidate parents, candidate parents to exclude for designated offspring, number of offspring per parent pair, number of runs, and duration of each run. Unlike CERVUS, COLONY can also infer genotypes of individuals that were not sampled and include those genotypes as candidate parents to assign offspring to missing individuals. COLONY also differs in how it determines confidence in an assignment: COLONY expresses its confidence in assignment by reporting probabilities for each assignment, which it calculates from the proportion of pedigree configurations that included that assignment out of the total number of pedigree configurations considered during a run. To be consistent with CERVUS, we considered any COLONY assignments with a probability of less than 0.8 to be made at a low confidence level and we considered these offspring unassigned.

For each simulated wood duck population, we ran four separate analyses in COLONY and CERVUS to assess the ability of the programs to assign offspring to their true mother when relatives are present in the candidate parent pool, males are

absent, and female sampling is incomplete. The configurations of the different analyses were chosen to explore the influence of specific aspects of missing information, but each configuration also represents a sampling scenario that applies to actual breeding systems studied to date (e.g. Eadie 1989; Forslund & Larsson 1995; McRae & Burke 1996; Ahlund & Andersson 2001). These configurations vary in whether parasites and/or males are included in the genetic samples. The first analysis served as a reference best-case scenario: we included all parents in the candidate parent pool, including paternal genotypes and the genotypes of all non-nesting brood parasites as well as nesting females in the population. Second, we excluded all males, but included both nesting and non-nesting parasitic females as candidate parents. Third, we included only nesting females and their mates as candidate parents, excluding parasitic females and their mates. Finally, we included nesting females and excluded males and parasitic females from the candidate parent pool. The role of relatives was included within each of these analyses, which included offspring from three categories of mothers: no relatives in the population, one half sibling sister, or one full sibling sister.

### 1.2.4. The Effect of Relatives and Number of Loci

We ran the maternity analyses described previously with populations that did not include relatives to compare to those with relatives included. We created populations without relatives by subsampling each of our original populations to exclude one half of each pair of related females. This resulted in a population of 48

candidate mothers, 48 candidate fathers, and 480 offspring. Of the 48 candidate mothers, 32 were nesting females, and 16 were parasitic females, preserving the ratio of nesting to parasitic females present in the original populations.

To explore how the number of loci affects the results of our analyses, we ran all of the analyses described above twice: once with the full set of 18 loci, and again with a set of 9 randomly selected loci (Appendix A1.1). For the reduced locus number analyses, we chose 9 loci because it is exactly half of the full set of loci and the average number of loci used to detect CBP and other alternative reproductive tactics, based on 28 studies conducted across bird taxa in the past 12 years (Table 1.2).

### 1.2.5. Program Parameters and Usage

For all analyses in both programs, we set the proportion of mothers or parents assumed sampled to reflect the actual proportion of mothers or parents included in the current run (0.67 for runs where non-nesting parasitic females were excluded, 1.00 for runs where all candidate parents were included). We chose to report an accurate proportion of mothers to present the best-case scenario under the conditions we were interested in investigating, as we have found that confidence level inflates with overestimates of the proportion of parents sampled, and deflates with underestimates of the proportion of females sampled, but does not change the identity of parents assigned (Appendix A1.2). For all COLONY analyses, we did not include any prior information on sibship among offspring or their parents and we allowed polygamy for both sexes, to match the parameters we used when working with our field sampled

genotypes and reflect the mating system in the natural populations (Bellrose & Holm 1994). Allowing polygamy in COLONY permits the program to consider that a candidate parent had multiple mates when constructing pedigrees but does not force the program to assign multiple mates per parent for the final parentage assignments, and so should not inaccurately report polygamous matings. We set COLONY to its longest processing time (as designated by the program) using the full likelihood approach to run four iterations of each analysis type to reduce sampling bias and maximize accuracy (Wang 2016).

### 1.2.6. Interpretation of Assignments and Errors

For each of the analyses in each program, we sorted offspring assignments into four categories: correctly assigned to the true mother, incorrectly assigned to a different female, correctly left unassigned (because its mother was not included in the sample), and incorrectly left unassigned (because the program lacked the ability to assign an offspring to its true mother at high confidence). The type of assignment error possible is dependent on the sampling context of the analysis and the tactic of the true mother of the offspring, so we report results in terms of the female's assigned reproductive tactic (nesting or parasitic). For females with relatives in the population, the programs could potentially incorrectly assign their offspring to a relative or a non-relative, and so we make the distinction between incorrect assignment to relatives and non-relatives in reporting these results. We averaged the proportion of offspring in each assignment error category across simulated populations, since there were not

many instances of population-level differences (Appendix A1.3). We used both the 80% confidence level and 95% confidence level as cut-offs for assignment to compare assignment errors based on confidence level.

In the analyses where we included either all candidate parents or all females, the best-case scenario would lead to the programs assigning 100% of the offspring (n=720) to their true mother at high confidence. For the analyses where we included only nesting parents or nesting females, the best case scenario would involve the programs being able to correctly assign the offspring of nesting parents to their true mother (accounting for 480 ducklings, or 67% of the total offspring), and correctly identify that the mothers of parasitic non-nesting females were absent from the candidate parent pool and so leave those offspring unassigned (accounting for 240 ducklings, or 33% of the total offspring).

## 1.3. RESULTS

### 1.3.1. Sampling Scenario

With all 18 loci, we were able to identify the true mother of most of the offspring regardless of the sampling scenario (Figure 1.2). Assignment errors were least frequent in the All Parent and All Female sampling scenarios, and most frequent in the Nesting Parents and Nesting Females scenarios (Figure 1.2). Overall, offspring of nesting mothers were more likely to be incorrectly unassigned and parasitic offspring were more likely to be incorrectly assigned. However, the magnitude and

type of assignment error varied within each scenario and was dependent on the program and the confidence level threshold we used.

Under the All Parent and All Female sampling scenarios, CERVUS rarely made mistakes in assigning offspring to their true mother (less than 1%). CERVUS struggled when we removed parasitic females from the candidate parent pool, resulting in higher assignment error rates in the Nesting Parents (6-7%) and Nesting Females (5-17%) scenarios. Most errors in the Nesting Parents scenario involved incorrectly unassigned offspring, with CERVUS incorrectly assigning only 1% or 2% of offspring. In the Nesting Females scenario, the absence of nesting fathers increased the proportion of incorrect assignments to between 4% and 17% of the offspring. All but one of the incorrectly assigned offspring were from parasitic mothers. CERVUS's tendency to incorrectly assign parasitic offspring when males and parasitic females are absent could result in underestimates of CBP and inflated estimates of reproductive effort for nesting females.

COLONY's parentage assignments were more robust to sampling context, likely because of the additional inference provided by the full pedigree approach of the program. Overall, COLONY was more likely to leave offspring incorrectly unassigned than incorrectly assign them. In the All Parents and All Females scenarios, COLONY did not incorrectly assign any offspring but left between 1% and 9% offspring incorrectly unassigned. These offspring were the progeny of both nesting females and parasitic females. When the candidate parent pool only included Nesting Parents or Nesting Females, COLONY incorrectly assigned less than 1% of

the offspring, and left up to 6% of the offspring incorrectly unassigned. The tendency of analyses in COLONY to leave offspring incorrectly unassigned could result in underestimates of CBP and reproductive effort but would not falsely inflate reproductive effort.

### 1.3.2. Programs and Confidence Level

The confidence level thresholds played an important role in producing the specific types of error we observed, especially in the Nesting Parents and Nesting Females scenarios. COLONY was able to accurately identify the correct mother of most of the offspring under any sampling context at the 80% level. When we used the 95% level as a threshold, many assignments that COLONY made correctly at the 80% confidence level were now categorized (incorrectly) as unassigned offspring. CERVUS assigned more offspring correctly at the 95% confidence level. At the 80% confidence level, offspring that had been unassigned at the 95% confidence level threshold were now assigned to the wrong mother.

Although COLONY often lacked the power to assign an offspring at high confidence, (resulting in unassigned offspring if the 95% cutoff is used), it nonetheless almost always indicated the correct mother in its assignments (Table 1.3). Even when COLONY did not have access to the parasite genotypes, it correctly identified parasitic offspring that belonged to the same missing mother, identified them as siblings, and indicated they were all progeny of a missing mother (with only a few exceptions when a nesting full sibling was in the candidate parent pool).

CERVUS is unable to infer missing genotypes, and so struggled more with parasitic offspring when we excluded their mothers from the candidate parent pool, sometimes resulting in high rates of incorrectly assigned parasitic offspring.

### 1.3.3. Number of Loci

When we reduced the number of loci available to the programs by half (n = 9), error rates increased dramatically, particularly in the All Females (10-45%) and Nesting Females (5%-25%) scenarios (Figure 1.3). This was mostly due to an increase in incorrectly unassigned offspring. As before, the error rate dropped with the inclusion of paternal genotypes in CERVUS only.

### 1.3.4. Relatedness

The exclusion of relatives from the candidate parent pool revealed that at least some of the patterns we observed in our runs with our original populations were driven by the presence of relatives (Figure 1.4,1.5). The presence of relatives in the population reduced the ability of both programs to assign offspring with high confidence (Figure 1.4). When we excluded relatives from the original populations, both programs left fewer offspring incorrectly unassigned, but CERVUS incorrectly assigned offspring at roughly the same rates as when relatives were present in the population. COLONY did not incorrectly assign any offspring when relatives were removed from the candidate parent pool.

When relatives were included in the candidate parent pool, candidate mothers with siblings in the population were most prone to having their offspring incorrectly assigned (up to 4%) or left unassigned (up to 7%), regardless of sampling context or confidence level cut-off (Figure 1.6A, 1.6B). When using CERVUS, incorrectly assigned offspring whose mothers had full siblings in the population were disproportionately assigned to the full sibling of the true mother. The only incorrect assignments COLONY made were between candidate mothers that were full siblings. The offspring left unassigned were primarily the progeny of females with siblings in the population, with the greatest number of unassigned offspring belonging to females with full siblings in the population. When we only included half the number of loci in the analyses, the errors were more proportionally distributed across the three relatedness categories (Figure 1.6C, 1.6D).

## 1.4. DISCUSSION

### 1.4.1. Sampling Context

Our brood parasitism simulations revealed that we generally succeeded in assigning parentage under the potentially challenging circumstances that we routinely observe in conspecific brood parasitic waterfowl, including a lack of male genotypes, unsampled parasitic females, and relatives in the population. Only a few sampling scenarios lead to substantial rates of assignment error, depending on the program and confidence threshold we used. The presence or absence of a mother in the candidate parent pool also determined the type of errors most likely to occur. The biological

implications of these assignment errors depend on the reproductive tactic of the mother of the offspring, the type of error made, the frequency of that error, and how it is interpreted (Table 1.1).

In our simulations, we found that analyses based on CERVUS/COLONY most frequently assigned the offspring of nesting females correctly. Consequently, estimates and patterns of nesting parasitism are likely accurate, especially if non-nesting parasites are included in the candidate pool (see below for complications arising from incorrect assignment of non-nesting parasitic offspring).

Offspring of females that were not included in the candidate pool were at the greatest risk of being incorrectly assigned to another female. As a result, non-nesting parasites are the most likely to go undetected or under-represented using genetic techniques alone. Non-nesting parasites have been reported to have the lowest reproductive success of all reproductive tactics in CBP (Ahlund & Andersson 2001; Jaatinen *et al.* 2011), but some of their offspring may have been falsely reported as belonging to a nesting female; our simulations suggest this would be especially problematic if researchers relied on CERVUS and an incomplete parent pool. Depending on what female the offspring of the non-nesting parasite are assigned to, incorrect assignment could lead to false patterns of CBP for nesting parasites and/or non-parasitic nesters (hosts).

False assignment of non-nesting parasitic offspring could also hinder our understanding of host responses to parasitism. In species such as the wood duck, which do not reject parasitic eggs, laying fewer eggs to compensate for a clutch size

enlarged by parasitism is an important trade-off and cost associated with parasitism (Andersson & Eriksson 1982; Eadie *et al.* 1988; Lyon 1998). Erroneous additions of non-nesting parasitic offspring to another female's reproductive effort could also result in misinterpretation of host laying responses to parasitism. If some parasitic eggs are erroneously attributed to their host mother, it may appear that the host mother either did not adjust her clutch size or possibly increased it in response to parasitism.

Finally, patterns of parasite host choice may be confounded if offspring of parasites without nests are attributed to females with nests (that may or may not be parasitic themselves). Host choice may provide insight into the costs and benefits that the two types of parasite are subject to by engaging in CBP. Nesting and non-nesting parasites are thought to be engaging in CBP under different ecological and physiological contexts and may be selecting hosts based on criteria that help mitigate these contexts. For example, previous theory and empirical studies have established that non-nesting parasites tend to be young, inexperienced, or physiologically incapable of nesting, characteristics which are associated with reduced clutch size and offspring survival (Sorenson 1993; Semel & Sherman 2001; Åhlund 2005; Nielsen *et al.* 2006; Lyon & Eadie 2008). Given these constraints, it may be critical for non-nesting parasitic females may seek out a more experienced host to increase the odds that their only offspring survive to hatch. Because nesting females have their own nest in addition to their parasitic offspring, their host selection criteria may be more relaxed or based on different criteria, If offspring of a non-nesting parasite are

incorrectly assigned to her nesting sister instead, a false pattern of parasitism would lead to a false conclusions about host choice for both parasite tactics and the costs and benefits associated with that choice of host.

It is important to note that while we did not explicitly incorporate nesting parasites into our simulation, the programs do not consider information about which nest each offspring came from. We chose to only have non-nesting parasites in our simulations, but we can infer how these programs would address nesting parasitism based on the assignment errors we observed in the offspring of the nesting females in our simulation. Genetic parentage assignment can accurately detect nesting parasitism since nesting offspring were rarely assigned to a nesting female that was not their true mother or left unassigned. The most likely case in which nesting parasitism may be under-reported is when offspring are incorrectly left unassigned due to a lack of power, which can be avoided with a sufficient set of loci.

### 1.4.2. Confidence Levels and Programs

To be able to directly compare results between COLONY and CERVUS, we imposed confidence level thresholds for COLONY based on CERVUS recommendations. However, it is important to emphasize that these two programs calculate and report confidence in assignment in very different ways, and the imposed confidence levels we used often changed how we interpreted assignments in COLONY. For example, COLONY assignments made at confidence levels lower than 80% were generally correct (Table 1.3) but with the arbitrary 80% confidence

level threshold these assignments were considered incorrectly unassigned. Few studies that use COLONY either report or impose a confidence level threshold (Table 1.2), and the fact that we did so to match CERVUS's assignment system meant that our interpretation of the apparent accuracy of COLONY assignments was likely conservative.

Our simulations show that COLONY and CERVUS were prone to different types of error. COLONY was more likely to leave offspring unassigned even when their mother was in the candidate pool (a false negative), and so potentially underestimates CBP and produces more muted differences among CBP tactics. CERVUS was more likely to incorrectly assign offspring of parasitic offspring to nesting females, and so artificially erase non-nesting patterns of CBP while potentially inflating nesting parasitism. Even with an arbitrary 80% threshold, we believe COLONY provide superior results, as the tendency to leave offspring unassigned rather than falsely assigned provides a more conservative estimate of CBP and minimizes incorrect patterns of CBP.

One unique strength of COLONY is its ability to assign offspring to a female it has inferred is missing from the population, which provides a picture of the reproductive effort and host choice of a unsampled female. While the offspring that COLONY identifies as being the progeny of an unsampled mother are technically left unassigned, we found that when we removed the parasitic females from the candidate parent pool, COLONY could correctly identify how many females were missing and assign the progeny of a missing female to a unique inferred missing female identity.

In other words, COLONY could identify a missing female's offspring and assign them back to her, without any information; this makes COLONY an especially useful in studies of CBP where many of the parasites may be unsampled.

The distribution of errors in terms of the individual females varied between programs. When COLONY made an error, it tended to incorrectly assign all the offspring of a particular female as a unit. When CERVUS made errors, it tended to incorrectly assign some, but not all, of a female's offspring. Consequently, the results from COLONY are more likely to underestimate the reproductive output of a few females, and where the results from CERVUS are more likely to underestimate the reproductive output of many females.

Our survey of genetic studies of CBP suggests that studies vary in the conditions we have found to affect error rates (Table 1.2). Many researchers rely solely on analyses conducted using CERVUS to draw conclusions about parentage and subsequent fitness proxies. Several studies that used CERVUS used an 80% confidence level cutoff combined with a fairly small number of loci, a combination that comes with a substantial risk of error, especially if unassigned offspring are considered parasitic offspring. Others have used an extensive set of loci (>15 loci) for assignment, which reduces the risk of incorrect lack of assignment regardless of the program used (Jaatinen *et al.* 2011; Šťovíček *et al.* 2013; Rodriguez-Martínez *et al.* 2014). Even with a 95% confidence level cutoff and an extensive set of loci, the error rates we observed in our CERVUS analyses could change the results of studies and their interpretation of results (see Lemons & Sedinger 2011; Indykiewicz *et al.* 2017).

Low rates of parasitism (5% or less) could be the result of or affected by assignment error rather than a true pattern in the system.

Given the potential incorrect assignment rates possible using CERVUS, we recommend that researchers instead use COLONY for future parentage analyses and consider running analyses previously done in CERVUS again using COLONY. We explored the possibility of using CERVUS as a secondary option to confirm lower confidence COLONY assignments, but found that although CERVUS assigned COLONY's low confidence assignments at high confidence, the rate of incorrect assignment was the comparable between the programs (Table 1.3). Accordingly, we suggest not imposing a confidence level threshold on COLONY assignments to eliminate incorrect lack of assignment, and minimizing incorrect assignments by proofing COLONY assignments with other lines of evidence from field studies (McRae & Burke 1996).

Both programs require allele frequencies as an integral part of their assignment process, so variance in population structure matters and results may vary between populations. While we did not observe consistent differences in assignment error that were unique to individual populations, we believe it is important to run simulations using allele frequencies that reflect the population of interest (Jones *et al.* 2010). Armed with simulations that reflect their unique study systems, researchers can identify the types of assignments that might be most prone to error and assess whether analyses lack the power to make assignments at all. Taking this additional

step will be necessary to enable researchers to more cautiously interpret the biological significance of their parentage assignments.

### 1.4.3. Number of Loci

In the runs with a reduced number of loci, offspring were incorrectly left unassigned more frequently than in the runs that included all 18 loci, regardless of the reproductive tactic of their mother. The inclusion of male genotypes in the candidate parent pool reduced both incorrect assignments and incorrect lack of assignments, apparently compensating in part for the lack of assignment power due to the reduced number of loci included in these analyses. The number of loci we used in the half loci analyses was the average number of loci (n=9) observed in recent avian CBP parentage assignment studies (Table 1.2), but the number of markers used to determine parentage varies widely between studies, and there is no clear consensus on a minimum number of markers for accurate assignments.

Our results strongly indicate that assignment accuracy and consequently reproductive estimates in alternative breeding systems can be substantially improved by including more than this average number of loci. In the past, some researchers have used lack of assignment to indicate that an offspring is the product of a parasitic event or extra-pair mating (Kreisinger *et al.* 2010; Lemons & Sedinger 2011; Tiedemann *et al.* 2011; Hario *et al.* 2012; Šťovíček *et al.* 2013), but our simulations show that even with 18 loci, unassigned offspring may be the progeny of parents present in the candidate parent pool. Future studies should not consider unassigned

offspring as parasitic offspring to obtain the most accurate estimates of CBP in their study populations. Previous studies should consider re-evaluating their conclusions after removing unassigned offspring as parasitic offspring, particularly if relatively few loci were used and/or the candidate parent pool was incomplete.

### 1.4.4. Relatedness

The presence of relatives in the breeding population increased the frequency of incorrectly unassigned offspring, indicating that high levels of kinship in a population can result in a loss of assignment power. While the most obvious risk with relatives in the population is incorrect assignment of an offspring to a relative of its true mother, our results suggests another less apparent risk from the presence of relatives in the population: increased incorrect lack of assignment. If these unassigned offspring are then considered parasitic offspring, the presence of kin in the population can cause an inflated estimate of parasitism rate, potentially leading to false conclusions about the importance of kinship in the evolution and dynamics of CBP. These problems can be avoided by not assuming that unassigned offspring are parasitic offspring.

With relatives present in the candidate parent pool and a full set of loci, offspring of mothers with siblings in the candidate parent pool were more susceptible to assignment error than those without relatives, although they were not necessarily incorrectly assigned to those relatives. Our results suggest that patterns of host-parasite relatedness are not exaggerated and kin-directed CBP is likely to go

underreported, particularly if the parasite does not have her own nest and/or was not sampled.

While incorrect assignments to relatives were comparatively rare, even small error rates could matter, particularly in the intersection of kin selection theory and CBP. In cases where the offspring of non-nesting females are incorrectly assigned to relatives, these errors exaggerate the reproductive success of their sibling, obscure any kin-directed non-nesting parasitism, and potentially create false patterns of nesting parasitism if their relative is a nesting parasite. Underestimating non-nesting parasitism directed at kin, and particularly full siblings as we observed most frequently in our simulations, means that we may be missing a vital aspect of the intersection of CBP and kin selection. When applied to CBP, kin selection theory (Hamilton 1964) suggests that the costs and benefits associated with laying parasitically in a relative's nest change depending on nesting status (Andersson 1984, 2001; Lyon & Eadie 2008; Eadie & Lyon 2011). Non-nesting parasites have no alternative to parasitic laying, and so have the most to gain from laying successfully in an altruistic relative's nest, as long as their indirect fitness does not decrease from the cost imposed on their related host. As such, related hosts may be a vital piece of the puzzle that enables the (even minor) success of non-nesting parasitism, and we may be misinterpreting the role of kin in CBP and missing important nuances that help explain the evolution of CBP if we cannot detect kin-directed non-nesting parasitism.

COLONY and CERVUS each have features designed to address kinship in populations. CERVUS allows the user to indicate what proportion of individuals in a candidate parent pool are related to one another, while COLONY allows users to input information about known relatedness between individuals. Both measures require an estimate of relatedness between candidate parents, but if a candidate parent is not sampled, as is often the case with non-nesting parasite females, it is impossible to calculate relatedness between the unsampled parent and another parent in the candidate parent pool. Since we were primarily interested in assessing how the programs performed when a subset of the breeding population was not sampled, we did not provide either program with an estimate of relatedness between individuals in any scenario. Our results suggest that program performance did not suffer due to this lack of information; we found that incorrect assignment rates between relatives were usually low (5% or less of the total offspring), and nearly absent when all females were included in the candidate parent pool.

### 1.4.5. Implications for past and future field studies

Our investigation into the accuracy of genetic assignment methods in CBP waterfowl revealed the overall strength of these approaches while highlighting a few specific weak points that could have important implications for how we interpret patterns of CBP. Although we designed our simulations to mirror scenarios found in CBP systems, our findings can be extrapolated to any system in which only a subset of the parents of interest are sampled and/or where the breeding population includes

first or second-order relatives. While these conditions are most frequently found in CBP and extra-pair paternity, they are also found in many parentage studies of natural populations outside of the realm of alterative mating systems. The patterns of assignment error in our simulations provide important insight into the pitfalls researchers may encounter in these systems as well.

One of the most important pitfalls we observed was reduced assignment ability leading to incorrectly unassigned offspring. Incorrect lack of assignment was most frequent in analyses with few loci and/or relatives in the candidate pool. These conditions arise frequently in CBP and EPP systems, where unassigned offspring are often considered the product of parasitism or extra-pair matings. Making this assumption comes with some risks, as our simulations show: the incorrectly unassigned offspring in our study were often the progeny of nesting parents that the programs, particularly COLONY, could not assign at high enough confidence. Incorrectly assuming these offspring were parasitic would inflate the frequency of parasitism erroneously and underestimate the reproductive effort of nesting mothers.

Another pitfall revealed by our simulations was an increase of incorrect assignments when the parent sample was incomplete and CERVUS was used. The Nesting Parent and Nesting Female sampling scenarios in CERVUS did produce incorrect assignments which could lead to inaccurate representations of reproductive effort, primarily for non-nesting parasites. Incorrect assignments of non-nesting parasitic offspring were especially common when we applied a lower confidence level threshold. This combination of factors could result in an underestimate of non-

nesting parasite reproductive effort and frequency of CBP and overestimate the reproductive success of nesting tactics in CBP. Additionally, incorrect assignment of parasitic offspring could erase kin-directed parasitism by non-nesting parasites, and even create spurious patterns of parasitism, depending on the identity of the falsely assigned mother.

Overall, the genetic parentage assignment tools researchers have employed to detect CBP perform well and allow researchers to calculate accurate estimates of reproductive effort, even when posed with the challenges associated with incomplete parent sampling and relatives in the population. Considering this finding, we encourage researchers to revisit their existing genetic datasets that they may have not viewed as detailed enough to use for investigations of parentage. Even without a complete sample of both sexes or the entire breeding population, existing datasets may be suitable for investigating patterns of parentage and alternative reproductive tactics, as even in the worst-case sampling scenarios programs, particularly COLONY, are usually able to correctly identify the true parent of an offspring.

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**Table 1.1.** The potential consequences of incorrect **assignments** and **lack of assignments** based on the type of error, the relationship of the false mother to the true mother is applicable, and which nest the offspring is located in. Note we only discuss consequences for offspring of non-nesting parasites, since those are the only parasites included in our simulations. We do not cover the consequences of incorrect assignment of the offspring of nesting females since this was a relatively rare occurrence in our simulations, particularly when all loci were included in the analyses.

| True Mother | Offspring in nest assigned to: | Result | Consequences | | | |
|---|---|---|---|---|---|---|
| | | | Frequency of CBP | Nester Reproductive Success | Parasite Reproductive Success | Kinship |
| Nesting | True nesting mother | Correctly assigned | Accurate | Accurate | – | – |
| Nesting | Parasite non-relative | Incorrectly assigned | Inflated | Underestimated | Inflated | – |
| Nesting | Parasite relative | Incorrectly assigned | Inflated | Underestimated | Inflated | Inflated |
| Nesting | None (Mother in sample) | Incorrectly unassigned | Inflated * | Underestimated | Inflated * | – |
| Nesting | None (Mother not in sample) | Correctly unassigned | Inflated * | Underestimated | Inflated * | – |
| Parasite | True parasite mother | Correctly assigned | Accurate | – | Accurate | – |
| Parasite | Host non-relative | Incorrectly assigned | Underestimated | Inflated | Underestimated | – |
| Parasite | Host relative | Incorrectly assigned | Underestimated | Inflated | Underestimated | Underestimated |
| Parasite | Other non-relative | Incorrectly assigned | Underestimated | – | Underestimated | – |
| Parasite | Other relative | Incorrectly assigned | Underestimated | – | Underestimated | Inflated*** |
| Parasite | None (Mother in sample) | Incorrectly unassigned | Underestimated ** | – | Underestimated | – |
| Parasite | None (Mother not in sample) | Correctly unassigned | Underestimated ** | – | Underestimated | – |

* if unassigned offspring are assumed to be parasitic, as some studies do
** if unassigned parasite offspring are excluded from the analysis
*** Kinship possibly inflated among parasite females

**Table 1.2.** Microsatellite based studies of CBP in birds, with methods and basic results summarized. NA indicates a value was not applicable because of the nature of the study, NR indicates that the value was not reported. Under the "Offspring Unassigned Parasitic" column, a C indicates that offspring were considered parasitic if unassigned but only under specific conditions, usually after applying another set of parental exclusion criteria. The minimum confidence level accepted for assignment is indicated in the "Min. CL" column.

| Species | Publication | System | Loci | Program | Percent Females Sampled | Percent Offspring Assigned | Percent Offspring Parasitic | Nesting Parasites | Non-nesting Parasites | Unassigned Offspring Parasitic | Min. CL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Barnacle goose** | Anderholm | CBP | 10 | CERVUS | 12 | NR | 12 | Y | Y | Y | NA |
| **Barrow's goldeneye** | Jaatinen | CBP | 19 | CERVUS | 60 | 80 | 13 | Y | Y | N | 95 |
| **Brant** | Lemons | CBP | 7 | CERVUS | NR | 100 | 6 | Y | NR | Y | NR |
| **Common eider** | Tiedemann | CBP | 7 | none | NA | NR | 17 | Y | Y | Y | NA |
| **Common eider** | Hario | CBP | 10 | COLONY | 38-65 | 100 | 34 | NR | NR | Y | NR |
| **Mallard** | Kreisinger | CBP, EPP | 8 | CERVUS | 70 | 100 | 10.1 | NA | NA | Y | 95 |
| **Mandarin duck** | Gong | CBP | 8 | CERVUS | 80 | 100 | 40.9 | Y | Y | C | 95 |
| **Common pochard** | Šťovíček | CBP | 17 | none | 100 | 80 | 39 | NA | NA | Y | NR |
| **Ruddy duck** | Reichart | CBP | 10 | CERVUS | NR | 89.00 | 29 | Y | Y | C | NR |
| **Wood duck** | Nielsen | CBP | 5 | none | NA | NR | 27 | NR | NR | C | NA |
| | | | | | | | | | | | |
| **Barn swallow** | Petrželková | CBP, EPP, QP | 6 | CERVUS, COLONY | 80 | 91.7 | 5.7 | Y | NR | Y | 95 |
| **Black-capped chickadee** | Otter | CBP, EPP,QP | 3 | CERVUS | 85 | 100 | 55 | NA | NA | N | 80 |
| **Black-headed gull** | Ležalová-Piálková | CBP, EPP | 6 | none | NA | NR | 9 | NR | NR | N | 95 |
| **Blue tit** | Griffith | CBP | 5 | CERVUS | 100 | 89 | 0 | NA | NA | NA | NR |

**Table 1.2 (continued).** Microsatellite based studies of CBP in birds, with methods and basic results summarized. NA indicates a value was not applicable because of the nature of the study, NR indicates that the value was not reported. Under the "Offspring Unassigned Parasitic" column, a C indicates that offspring were considered parasitic if unassigned but only under specific conditions, usually after applying another set of parental exclusion criteria. The minimum confidence level accepted for assignment is indicated in the "Min. CL" column.

| Species | Publication | System | Loci | Program | Percent Females Sampled | Percent Offspring Assigned | Percent Offspring Parasitic | Nesting Parasites | Non-nesting Parasites | Unassigned Offspring Parasitic | Min. CL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Burrowing owl** | Rodriguez-Martínez | CBP, EPP | 17 | CERVUS | 100 | 100 | 5.7 | NR | NR | NA | 80 |
| **European roller** | Sánchez-Tójar | CBP, EPP, QP | 6 | CERVUS | 75 | 54 | 0 | NA | NA | Y | 95 |
| **Florida scrub-jay** | Peer | CBP | 7 | CERVUS | NR | 0 | NR | N | N | NR | NR |
| **Houbara bustard** | Lesobre | CBP | 12 | CERVUS | 80 | 73 | 26 | N | Y | Y | NR |
| **Imperial shag** | Calderón | CBP, EPP | 4 | none | NA | NA | 0 | NA | NA | NA | NA |
| **Monk parakeet** | Jose Martinez | CBP, EPP | 7 | COLONY | 100 | NR | 1.2 | NR | NR | Y | NR |
| **Northern bobwhite** | Davis | CBP | 12 | COLONY | 95 | 100 | 21 | NR | NR | N | 80 |
| **Northern flicker** | Wiebe | CBP, EPP | 12 | CERVUS | 95 | 100 | 5 | Y | NR | Y | 95 |
| **Prothonary warbler** | Tucker | CBP | 6 | CERVUS | 92.5 | NR | 12.5 | Y | Y | NR | 95 |
| **Sage grouse** | Bird | CBP, MP | 13 | CERVUS | 20-90 | 47.6 | 2.2 | NR | NR | Y | 80 |
| **Song sparrow** | Latif | CBP | 10 | CERVUS | 100 | 100 | 0 | N | N | NA | NR |
| **Valais hoopoe** | Berthier | CBP, EPP | 6 | CERVUS | 60 | 71 | 7 | NR | NR | C | 80 |
| **Zebra finch** | Schielzeth | CBP | 10 | none | 100 | | 5.4 | Y | NA | NR | NR |

**Table 1.3.** The average number of incorrect assignments made by COLONY for offspring assigned at confidence levels under 80% by the program, compared to CERVUS' incorrect assignment rate for the same offspring. These numbers come from our analyses with all 18 loci. We calculated the number of incorrect assignments in confidence level intervals of 10%. We only report intervals that contained errors for each analysis type. Note that we do not report confidence levels for the CERVUS assignments, and so they were not necessarily assigned at a confidence level lower than 80%.

| Analysis | Confidence level interval | Average # of assignments in confidence level interval | Average # COLONY incorrect assignments | Average # CERVUS incorrect assignments |
|---|---|---|---|---|
| **All Parents** | 10-20% | 10 | 1.5 | 1 |
| **All Females** | 30-40% | 5 | 0 | 0.5 |
| **Nesting Parents** | 70-80% | 10 | 5 | 5 |
| **Nesting Females** | 70-80% | 10 | 0 | 5 |

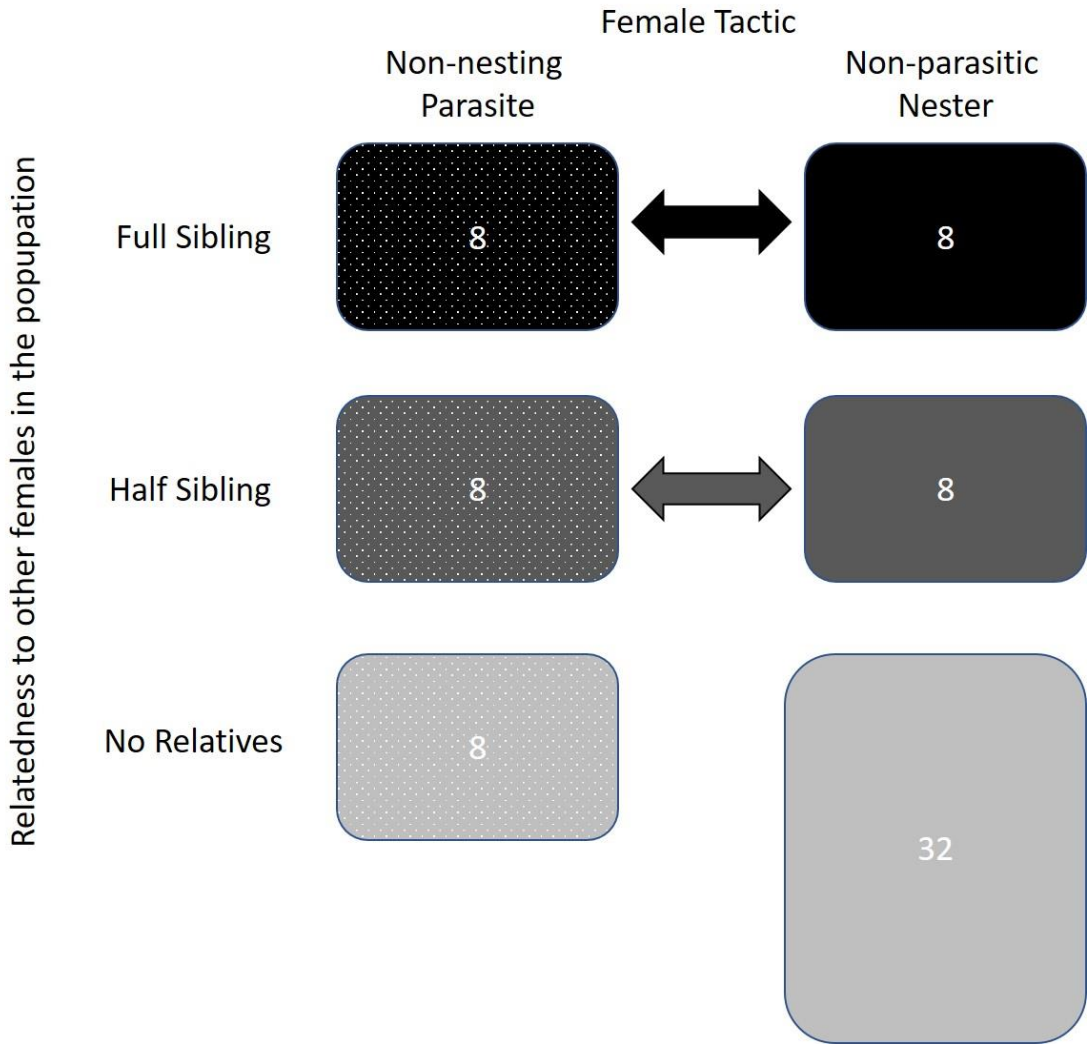


**Figure 1.1.** Overview of relatedness and nesting status of simulated second-generation (G2) females (n = 72). The relatives were divided between the two breeding tactics: non-nesting parasite and non-parasitic nester. There were eight pairs of full siblings with one member in each of the two breeding categories; the same pattern applies to the half siblings. The remaining females did not have relatives in the population, and were divided between non-nesting parasites (n=8) and non-parasitic nesters (n=32).

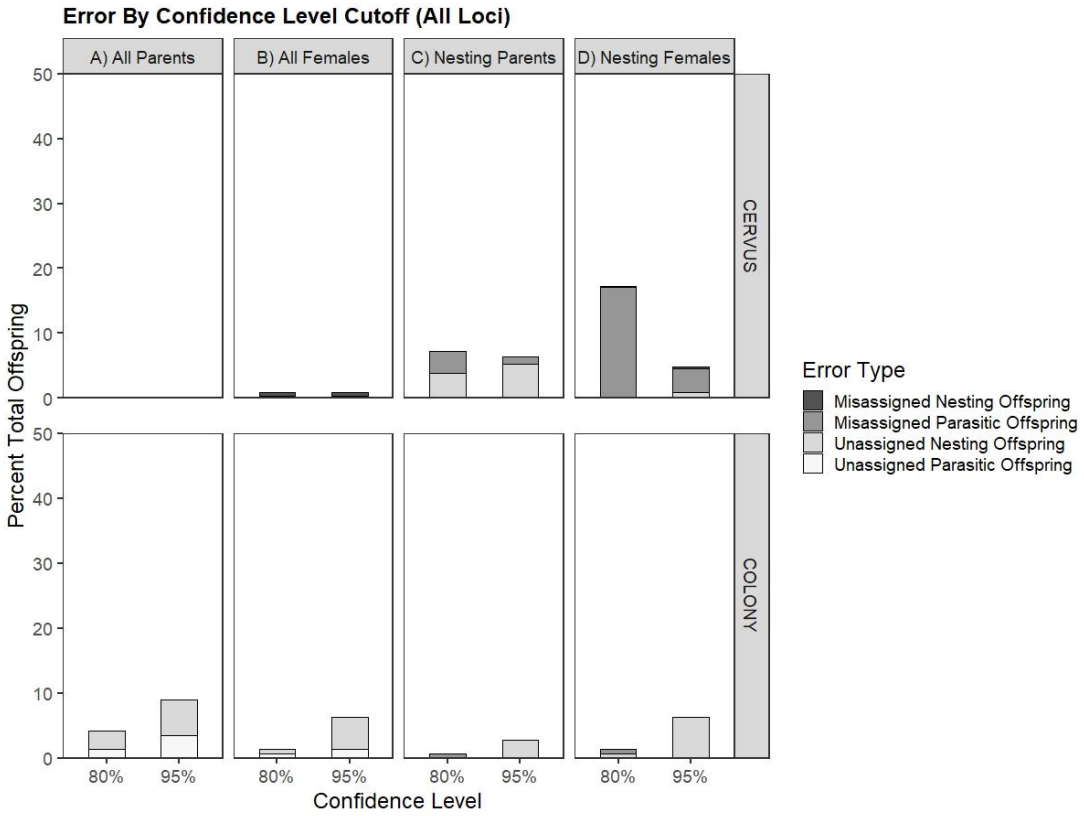


**Figure 1.2.** Comparison of the effect of four sampling contexts on the proportion of offspring correctly and incorrectly assigned or left unassigned by the programs COLONY and CERVUS using either a 80% or 95% confidence level cut-off. In A) All candidate parents were included in the analysis, including parasitic females and their mates. B) All male genotypes were excluded, so that only females were in the candidate parent pool. C) Parasitic females and their mates were excluded from the parent pool, so that only nesting females and their mates were candidate parents. D) Parasitic females and all males were excluded from the candidate parent pool, so that only nesting females were included. Indicated for each analysis is the percent of the 720 total offspring in each of four categories based on nesting status of the mother and error type: 1) misassigned offspring of nesting parents, 2) misassigned parasitic offspring, 3) unassigned offspring of nesting parents, 4) unassigned parasitic offspring. These analyses included all 18 loci.

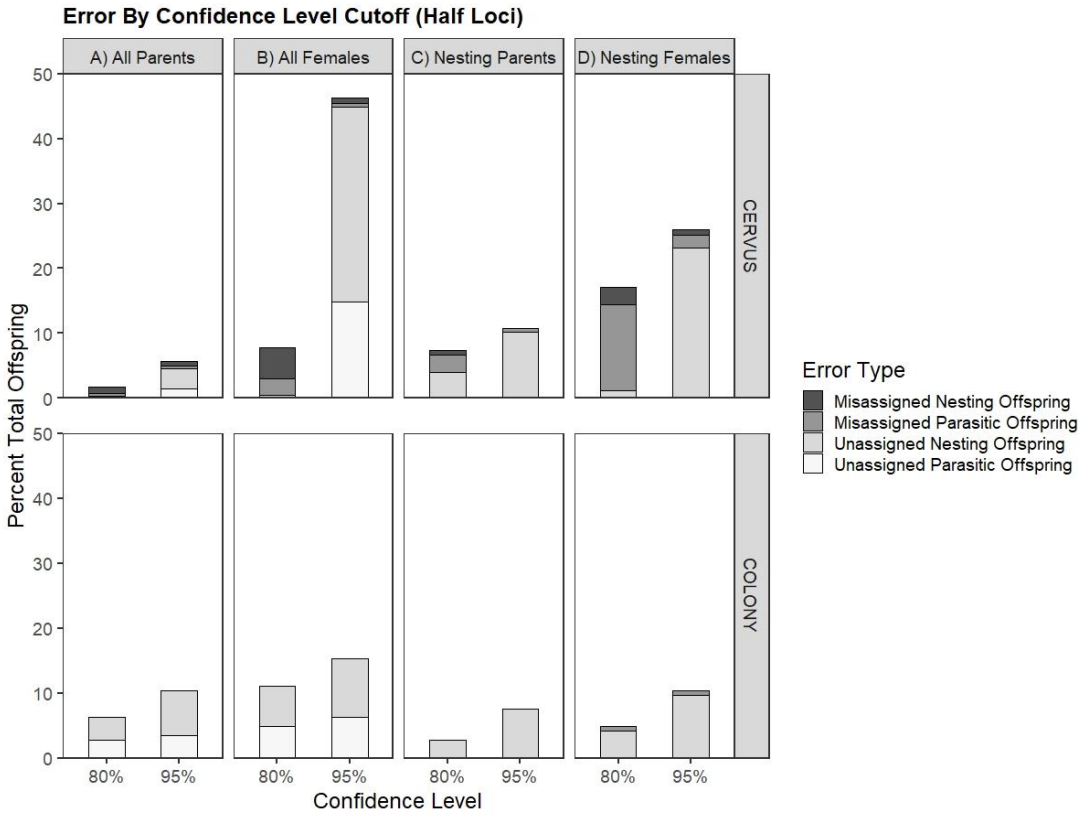


**Figure 1.3**. Comparison of analysis types where a subset of 9 loci were included, presented with either a 80% or 95% confidence level cut-off. In A) All candidate parents were included in the analysis, including parasitic females and their mates. B) All male genotypes were excluded, so that only females were in the candidate parent pool. C) The parasitic females and their mates were excluded from the parent pool, so that only nesting females and their mates were candidate parents. D) Parasitic females and males were excluded from the candidate parent pool, so that only nesting females were included. Shown are errors in assignment sorted by the reproductive tactic of the mother, so offspring are either 1) misassigned offspring of nesting parents, 2) unassigned offspring of nesting parents, 3) misassigned parasitic offspring, 4) unassigned parasitic offspring.

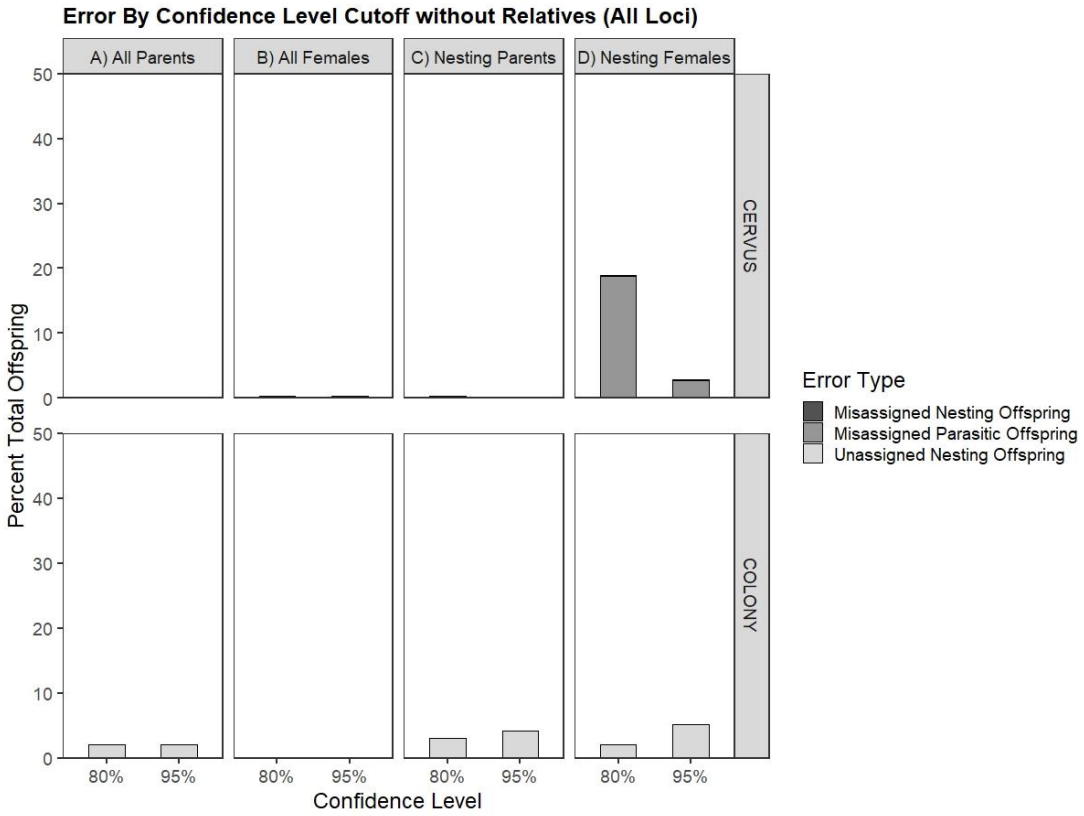


**Figure 1.4.** Comparison of the effect of four sampling contexts on the proportion of offspring correctly and incorrectly assigned or left unassigned by the programs COLONY and CERVUS **without relatives** (n = 480) using either a 80% or 95% confidence level cut-off. These analyses included all **18** loci. In A) All candidate parents were included in the analysis, including parasitic females and their mates. B) All male genotypes were excluded, so that only females were in the candidate parent pool. C) Parasitic females and their mates were excluded from the parent pool, so that only nesting females and their mates were candidate parents. D) Parasitic females and all males were excluded from the candidate parent pool, so that only nesting females were included. Indicated for each analysis is the percent of the 480 total offspring in each of four categories based on nesting status of the mother and error type: 1) misassigned offspring of nesting parents, 2) misassigned parasitic offspring, 3) unassigned offspring of nesting parents, 4) unassigned parasitic offspring.

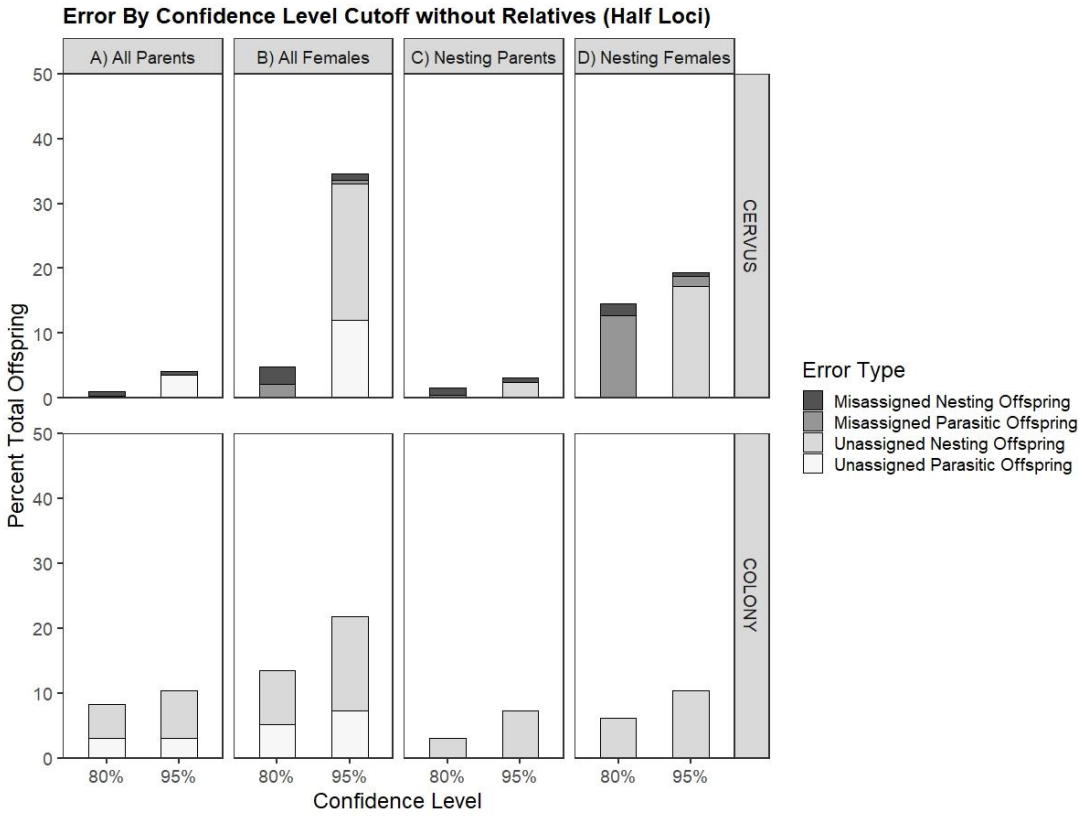


**Figure 1.5.** Comparison of the effect of four sampling contexts on the proportion of offspring correctly and incorrectly assigned or left unassigned by the programs COLONY and CERVUS **without relatives** (n = 480) using either a 80% or 95% confidence level cut-off. These analyses included **9** loci. In A) All candidate parents were included in the analysis, including parasitic females and their mates. B) All male genotypes were excluded, so that only females were in the candidate parent pool. C) Parasitic females and their mates were excluded from the parent pool, so that only nesting females and their mates were candidate parents. D) Parasitic females and all males were excluded from the candidate parent pool, so that only nesting females were included. Indicated for each analysis is the percent of the 480 total offspring in each of four categories based on nesting status of the mother and error type: 1) misassigned offspring of nesting parents, 2) misassigned parasitic offspring, 3) unassigned offspring of nesting parents, 4) unassigned parasitic offspring.

**Figure 1.6 .** The distributions of errors across three kin-based categories. Each graph displays a different loci number and confidence level cut-off combination: A) displays all loci and 95% confidence level, B) displays all loci and 80% confidence level. Candidate mothers can have full siblings (FS), half-siblings (HS), or no relatives (NR) in the population. Error values are averaged across the two simulated populations.

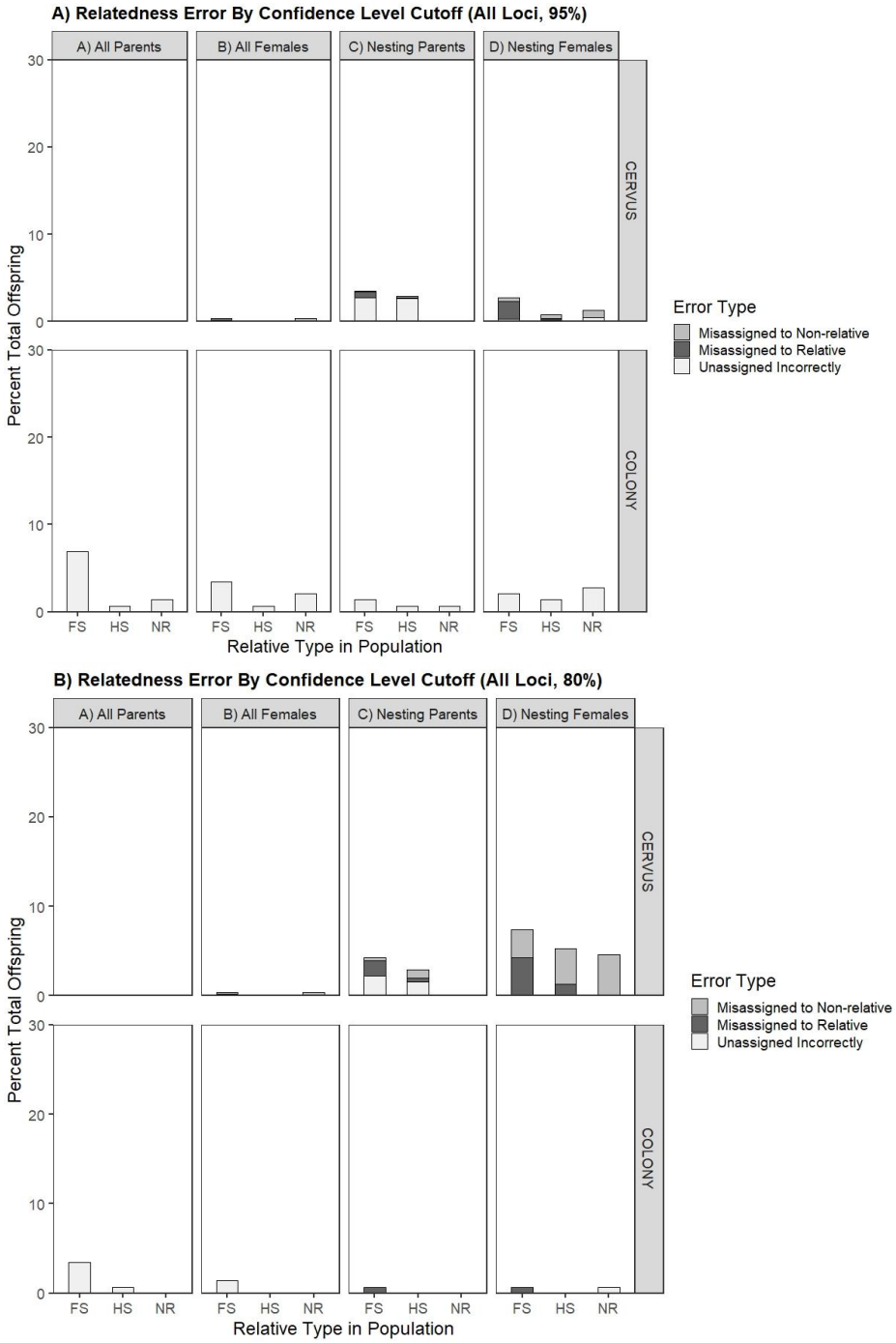


**Figure 1.6.** The distributions of errors across three kin-based categories. Each graph displays a different loci number and confidence level cut-off combination: A) all loci and 95% confidence level, B) ) all loci and 80% confidence level, C) ) half loci and 95% confidence level, D) half loci and 80% confidence level. Candidate mothers can have full siblings (FS), half-siblings (HS), or no relatives (NR) in the population. Error values are averaged across the two simulated populations.

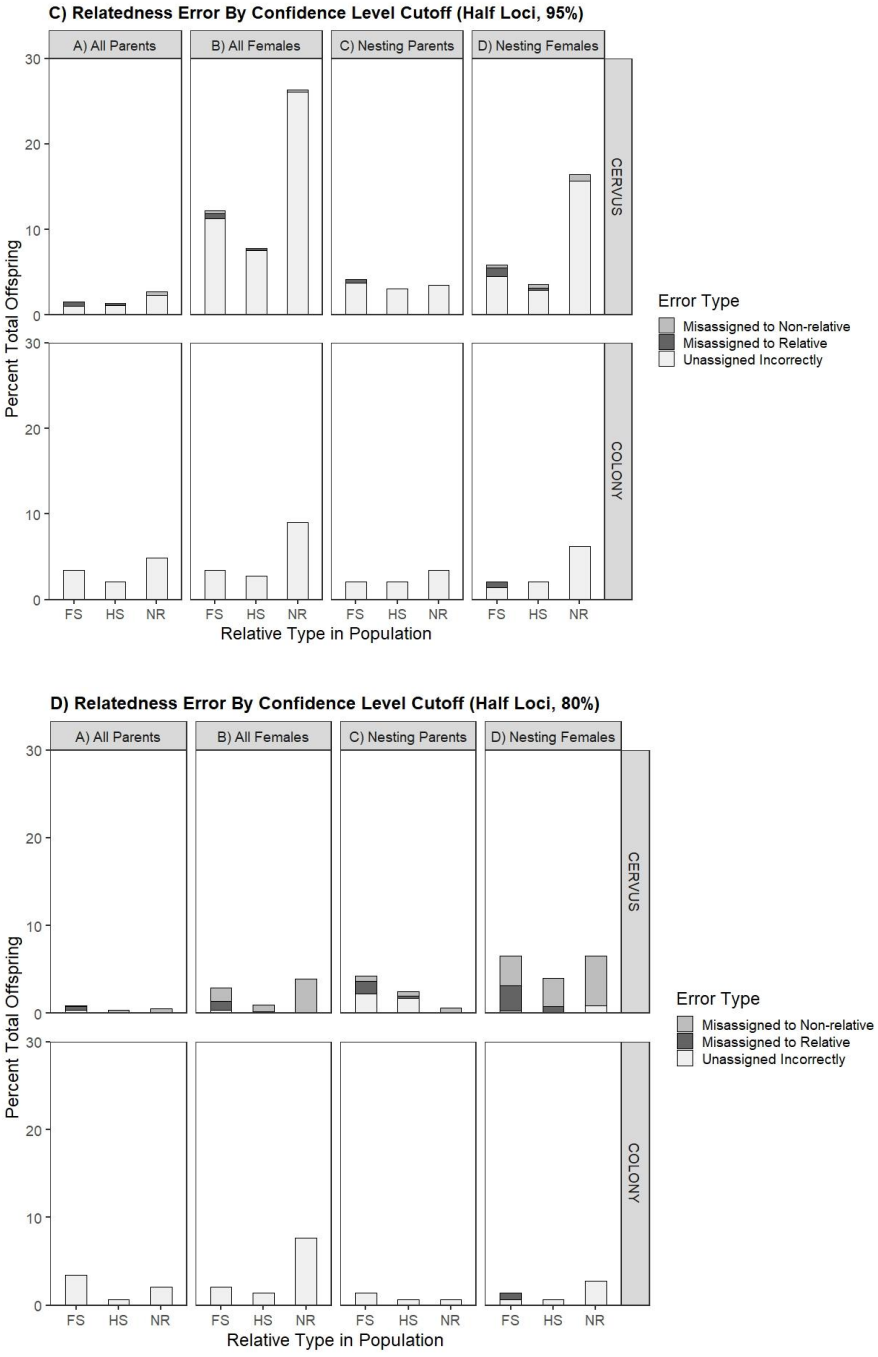


**Figure 1.6 (continued).** The distributions of errors across three kin-based categories. Each graph displays a different loci number and confidence level cut-off combination: C) displays half loci and 95% confidence level, D) displays half loci and 80% confidence level. Candidate mothers can have full siblings (FS), half-siblings (HS), or no relatives (NR) in the population. Error values are averaged across the two simulated populations.

# CHAPTER 2: Leveraging genetic data with non-genetic information to assess parentage in systems with alternative reproductive tactics

## 2.1. INTRODUCTION

The measurement of fitness is central to all evolutionary biology and, depending on the question of interest, is measured in terms of survival, mating success, or reproductive success (Clutton-Brock 1988). Reproductive success is fundamental to understanding sexual selection, kin selection, mating systems, reproductive tactics and strategies, and the evolution of social behaviors generally. To estimate reproductive success accurately it is essential to correctly identify the parentage of all offspring in a population. Historically, researchers assumed that the individuals that raise an offspring (the social parents) are also the genetic parents (Coltman *et al.* 1999). However, many organisms have complex breeding systems that result in alloparental care, where individuals end up caring for offspring that are not their genetic offspring (Petrie & Møller 1991; Scott & Williams 1994; Lyon & Eadie 2008). For example, such parentage can result from females mating with multiple males, cooperative breeding, or alternative reproductive tactics like conspecific brood parasitism (CBP). While assigning maternity is often more straightforward than assigning paternity due to closer associations between mothers and offspring in many taxa, female alternative reproductive tactics (ARTS) can result in offspring from more than one female associating with an apparent mother (Alonzo & Klug 2012).

Traditionally, assigning parentage in systems with cryptic parentage relied on direct behavioral observations and clues from the natural history of study organisms (e.g. maximum clutch size, shared nest site, egg laying rate, shared morphology) to restrict possible parental candidates or directly assign parentage (Westneat *et al.* 1987; Griffith *et al.* 2002; Eadie *et al.* 2010). Gathering the observations required to accurately assign parentage in ARTS systems can be challenging, as observing all individuals for a complete breeding season is prohibitively expensive and requires extensive hours of field work to obtain direct evidence of individual breeding events (Coltman *et al.* 1999). In addition, non-genetic techniques cannot always provide estimates of fecundity, reproductive success, and behavior at the level of the individual parent as these methods are often unable to connect offspring directly to their parents (Westneat *et al.* 1987).

With the advent of genetic parentage assignment, researchers have been able to gain insight into reproductive behavior that would have been nearly impossible using only traditional non-genetic methods, ranging from the individual fecundities of parents to population-level frequency of breeding tactics (Scott & Williams 1994; Hughes 1998; Avise *et al.* 2002; Griffith *et al.* 2002). However, genetic techniques are inherently based on genotype frequencies and so the strength of assignments is dependent on several factors, including the quality of the genotypes involved in the analysis, the number of markers available, and the composition of the candidate parent pool (Jones & Ardren 2003; Araki & Blouin 2005; Jones *et al.* 2010; Harrison *et al.* 2013; Lemons *et al.* 2014). To reinforce genetic assignments, researchers often

use other genetic-based criteria, such as the number of mismatches between a candidate parent and its putative offspring, or minimum confidence level requirements for parental matches (Jones & Ardren 2003). These criteria have their own limitations; for example, confidence levels are probability based, and intrinsically include accepting a level of error (e.g. accepting assignments above an 80% confidence level includes a 20% error rate). Additionally, as these supplemental criteria originate from the same genetic data as the assignments they are attempting to verify, they are not an independent source of information for corroboration of parentage.

Despite non-genetic parentage assignment methods falling out of favor with the rise of genetic assignment, there is still debate over the value of ecological information to refine genetic assignments. Many researchers rely exclusively on genetic information, citing the apparent improvement in parentage studies that result from genetic assessments instead of pure non-genetic estimates (Griffith *et al.* 2002; Grønstøl *et al.* 2006). Others argue ecological data is essential for approximating patterns of parentage accurately (Sorenson & Payne 2002; Eadie *et al.* 2010). This debate is worth revisiting in the light of recent advances in remote sensing tools such as radio frequency identification (RFID, Bonter & Bridge 2011) and GPS tagging (Bairlein 2003; Bridge *et al.* 2013), which provide a wealth of behavioral observations without requiring extensive field observations. Although remote monitoring systems are initially expensive to invest in and do not provide direct observations of specific breeding activities, the sheer volume of behavioral data

collected can provide insight into patterns of breeding behavior that can help narrow the pool of feasible candidate parents. For example, remote sensing data has the potential to refine genetic assignments by recording the presence of a potential parent during critical times in reproduction, such as timing of egg laying in a focal nest. However, the extent to which remote monitoring systems can actually improve parentage assessment remains unclear because the technology has only recently been integrated into ecological studies (Bridge *et al.* 2019).

Conspecific brood parasitism (CBP) is a female ART that results in multiple maternity broods and hence provides an excellent system for testing the concordance of genetic assignments with non-genetic sources of parentage information. CBP occurs when females lay eggs in the nests of other females of the same species and provide no subsequent parental care. These mixed parentage nests create uncertainty in offspring maternity, so accurate assignment of offspring in a nest is crucial for identifying parasitic offspring, determining the reproductive options females choose, and detecting parasitism at the population level. Females in CBP systems may engage in one of three tactics during a breeding season: 1) establishing their own nests without laying parasitically, 2) laying parasitically in addition to establishing their own nest, or 3) only laying parasitically. Although CBP has been investigated for decades, researchers are just beginning to uncover the behavioral patterns and fitness consequences that may explain the evolution and maintenance of CBP tactics (Lyon & Eadie 2018). Accurately determining the frequency and reproductive success of each tactic is a critical first step in understanding both the adaptive basis of CBP and

the link between the behavior and population dynamics (Eadie & Fryxell 1992; Nee & May 1993; de Valpine & Eadie 2008).

Researchers have used a variety of non-genetic methods to detect the frequency of CBP tactics at the population level (Eadie *et al.* 2010): direct observation of egg-laying female activity, frequency and timing of egg laying, and abnormal clutch sizes provide an estimate of population-level frequency of parasitism and in some cases can even identify parasitic females. However, these techniques often fail to specifically pinpoint which offspring was produced by which female. Consequently, these approaches not only underestimate parasitism rates, but they also make it impossible to assess fitness gained from different reproductive tactics since they cannot estimate fecundity and reproductive success reliably.

Without accurate parentage assignments researchers risk painting an incomplete or inaccurate picture of individual fitness, making it very difficult to understand how behaviors like CBP evolve and function at multiple levels. From a broader evolutionary perspective, comparative studies rely on population level estimates of CBP (e.g. Yom-Tov 2001), which, if incorrect, makes it more difficult to understand the broad evolutionary patterns and correlates of the behavior. At the individual level, inaccurate assignments could lead to erroneous estimates of tactic frequency, fecundity, and success, resulting in muted patterns that could be hard to distinguish from error, and muddy conclusions regarding the adaptive basis of behaviors. For example, underestimating the fitness of nesting parasite tactics by incorrectly assigning parasitic offspring to their host could lead to the conclusion that

nesting parasites are less successful than their non-parasitic counterparts. Even our perception of fine-tuned behavioral interactions, such as choice of host, could be swamped out if parasitic offspring are not detected accurately.

Here, we investigate how various non-genetic methods of detecting CBP compare to genetic methods at the population level, and if validating genetic assignments with behavioral RFID observations provides an independent check of genetic estimates of CBP at the individual level in a wild population of wood ducks (*Aix sponsa*). We answer three questions: 1) How concordant are genetic estimates of CBP tactics with non-genetic estimates at the population level? 2) Can RFID evidence help refine estimates of individual tactics and fecundities made through genetics? 3) Does RFID evidence support the concept that low confidence genetic assignments have a higher risk of error? We describe the frequency of CBP in wood ducks at the population and individual tactic level by comparing evidence from genetics alone, genetics and traditional non-genetic methods of detecting CBP, and genetics combined with RFID. The extensive dataset we use for this study combines over 1100 genotypes and thousands of direct nest observations and RFID reads, making it ideal to assess the utility of integrating non-genetic information and genetic assignments in CBP. Additionally, this study builds on and provides practical context for our earlier study of genetic techniques in CBP, which indicated that genetic methods assign parentage accurately in our system but was entirely reliant on simplified simulations based on field-collected genotypes (Thow *et al.* manuscript).

## 2.2. METHODS

### 2.2.1. Study Species

Wood ducks are cavity nesters that readily nest in nest boxes, with an average clutch size of 12 eggs and a range from 1 to 58 eggs in our study populations (Odell & Eadie 2010). Previous studies indicate that wood ducks are likely polygamous or seasonally monogamous, but their mating system has yet to be conclusively determined (Bellrose & Holm 1994; Baldassarre 2014). Male wood ducks attend females during nest site selection and mating but provide no parental care. Wood ducks are unique among North American waterfowl in that they can produce a second clutch after their first one fledges (Bellrose & Holm 1994); as such, nest boxes may reused in a season, producing multiple nesting attempts per box.

### 2.2.2. Field Methods

From 2012 to 2016, we monitored female breeding activity at three sites in Yolo County, CA: Conaway Ranch in Woodland, Roosevelt Ranch in Zamora, and Russell Ranch in Davis. We visited nests to assess the status of the current attempt two to three times a week at Conaway Ranch and Russell Ranch, and weekly at Roosevelt Ranch. At each visit, we counted and marked new eggs with a permanent felt-tip marker. For this study, we use nesting data from the 188 wood duck nest boxes we monitored during the 2016 breeding season, as it was a high effort year with the most comprehensive genetic and field data coverage.

To obtain the genotypes used for genetic assignments, we caught nesting females on their nests between the first and third week of incubation to band them and collect a blood sample. To sample non-nesting females that could have been actively breeding, we made additional efforts to capture unbanded females during and after the breeding season by deploying nest traps and bait traps, which are designed to capture foraging ducks away from the nest. We did not include male genotypes in the study because males are not associated with nests and cannot be consistently trapped and sampled. All offspring were sampled at hatch, either by taking a blood sample or collecting eggs that did not hatch for later dissection. We sampled offspring from 103 of 126 nesting attempts in 2016. Further details on blood sampling, DNA extraction, and genotyping are provided in Chapter 1 (Thow *et al.* manuscript)

Starting in 2014, we tracked activity of breeding individuals at nest boxes by inserting RFID tags between the scapula of all individuals during their initial handling either as a duckling or an adult. We used 12-gauge implanter needles developed by Biomark, and passive 2x12mm (125 kHz) tags from Cyntag. We fitted each nest box entrance with a battery-operated RFID reader (Bridge *et al.* 2019) to record entrances and exits of RFID tagged individuals. Previously banded birds encountered during trapping or nest checks were scanned to ensure their RFID tag was in place. We also replaced batteries and downloaded RFID reads once a week to ensure continuous data collection and operation. We were able to collect RFID reads from 98 of the 103 nesting attempts we obtained genetic samples from (the remaining 5 nests did not have operating RFID units during the 2016 season).

### 2.2.3. Genetic Analysis

We used COLONY (Version 2.0.6.4, Jones & Wang 2010) to assign offspring hatched in 2016 to candidate mothers. COLONY assigns offspring to a candidate mother and calculates a probability associated with each assignment. COLONY assigns all offspring to a mother; all offspring not assigned to an actual candidate mother in the pool of included genotypes are assigned to inferred genotypes of unsampled females. Each individual was genotyped at 19 microsatellite loci developed for wood ducks and other closely related taxa (Odell 2008). We included genotypes from females sampled prior to and during 2016 so that we could assign offspring to females that may have laid eggs parasitically without nesting or being trapped in 2016. Although wood duck females display natal philopatry and thus may nest near and/or parasitize nesting relatives, we have established that there is no risk of incorrectly assigning an offspring to a relative using COLONY (Thow *et al*. manuscript). For all analyses, we allowed polygamy, presumed a female sampling rate of 70%, and did not include any prior information on sibship among offspring or candidate mothers. We set COLONY to its longest processing time (as designated by the program) using the full likelihood approach to run four iterations of each site-specific analysis to reduce sampling bias and maximize accuracy (Wang 2016).

### 2.2.4. Population Level Detection of CBP

To assess the level of concordance between genetic and non-genetic estimates of CBP at the population level, we compared the genetic assignments from COLONY

to lines of non-genetic evidence used in previous studies to assess CBP in birds, including wood ducks: egg accumulation rate (Jackson 1992; McRae 1997; Lyon 2003a; Semel & Sherman 2007), presence of new eggs after the laying period (Clawson *et al.* 1979; Romagnano *et al.* 1990; Robertson *et al.* 1992; Lyon 1993a), and abnormal clutch sizes (Dugger & Blums 2001; Yom-Tov 2001; Rohwer & Heusmann 2007; Roy *et al.* 2009). We also compared behavioral evidence from RFID reads to genetic estimates of CBP; we do not believe this has been done previously in any study of CBP. RFID visitation cannot by conclusively tied to egg laying activity, as wood ducks are known to prospect nests before laying eggs in them (Bellrose & Holm 1994); however, RFID activity can provide potentially useful support for genetic estimates of parasitism by establishing if a parasite was present when a parasitic egg was laid in a nest.

To obtain genetic estimates of CBP, we summarized genetic assignments to determine if a nest was parasitized and how many females were assigned offspring in a nest. If one offspring was assigned to a female other than the host female, we considered that nest parasitized using the genetic criterion.

To determine CBP estimates using the egg accumulation criterion, we calculated the daily egg accumulation rate to determine if an offspring in that nest could have been produced by a parasite. Waterfowl lay at most one egg per 24-hour period (Drobney 1980), so the appearance of 2 or more eggs a day during the laying period indicates a parasitic event (Lyon 1993b; McRae 1997). The appearance of a new egg after the laying period has concluded is also an indication of parasitism

because females do not typically lay eggs once they start incubating. The laying period for a nest began on the day the first egg was laid. Parasitism can make it difficult to determine when the host's laying period ends because eggs continue to accumulate after the host has ceased laying. We therefore estimated the end of the laying period by working backwards from hatch. Wood duck eggs develop for between 28-32 days (Bellrose & Holm 1994), so we defined the end of the laying period as 32 days before the nest started to hatch. If at any point in the laying period the average number of eggs that accumulated in 24 hours was greater than one, and/or if any eggs were added after the onset of incubation (calculated as the day after the end of the laying period), we considered that nest parasitized. We recorded the maximum number of eggs accumulated in 24 hours to assess if it had a relationship to the number of parasitic eggs deposited in a nest. We could not calculate egg accumulation rates for 30% (n=31) or determine if any eggs were laid during incubation for 34% (n=35) of the nests in our study due to infrequent visits to those nests.

We also assessed CBP in our populations using a range of clutch size cutoffs. Early studies of wood ducks determined that clutch sizes exceeding 15-17 eggs were likely dump nests (Morse & Wight 1969; Haramis 1975; Clawson *et al.* 1979) based on evidence from egg accumulation rates and the presence of unhatched eggs. In contrast, Semel and Sherman (2007) indicated that a clutch size cutoff of 12 eggs would most closely match true parasitism rates in wood ducks, as determined by egg accumulation rates. To assess CBP estimates using clutch size, we calculated the

percentage of nests parasitized according to multiple clutch size cutoffs, ranging from 10 eggs, which could overestimate parasitism, to the most conservative option of 22 eggs (Bellrose & Holm 1994), which is likely to underestimate parasitism.

To assess CBP at the population level using RFID, we analyzed RFID records to determine the number of unique females that were logged on a given nest during the entire nesting attempt, from nest initiation to the day before the nest hatched or all eggs were collected due to nest failure. We considered the nest parasitized if the number of RFID logged females was greater than one. This assumption comes with two caveats: wood ducks prospect nests before laying eggs, so not every RFID logged visit is from a female laying an egg. By making this assumption, we may be frequently overestimating parasitism rates. Additionally, we cannot detect parasitism from females that have not been RFID tagged, which may cause us to underestimate parasitism in some cases. However, RFID estimates of CBP at the population level are still valuable in combination with estimates of CBP derived from other methods, as nests that are apparently parasitized according to other criteria should be supported by visits from multiple RFID tagged females. We also compared female visitation from RFID logs to the number of genetically detected parasitic eggs to determine the relationship of female visitation to parasite activity.

We estimated the total number of parasitic and non-parasitic (host) eggs produced in our populations by summarizing offspring into those categories using genetic and RFID evidence both together and in isolation. All other non-genetic methods of determining CBP we considered are unable to directly tie a female to their

offspring and so cannot estimate how many parasitic eggs were produced in a population.

### 2.2.5. Individual Tactic Determination and Fecundities

RFID reads can be used to assess individual tactics and fecundities as determined by genetics, as they can tie an individual female to a nest. Specifically, if both genetics and RFID are fully accurate, any female detected as a brood parasite at a nest through genetics should also be detected as visiting the nest by RFID. To assess this prediction, we determined if the genetically-assigned parasite was logged on the nest during the time a parasitic egg would have been laid in the nest. For hatched offspring, this time period was the laying period. For unhatched offspring, we estimated the age of the embryo in days during dissection, and then subtracted that number of days from the date the nest hatched. If the female was present on the nest during the week the egg was laid, then we considered her genetic assignment supported by RFID evidence. We compared the tactics determined by genetics alone to the tactics as determined by RFID and genetics in combination. We also assessed if incorporating RFID evidence changed the number of offspring that could be attributed to a female, by comparing the number of genetically-assigned offspring to the number of genetic offspring that RFID also indicated she could have produced.

### 2.2.6. Genetic Assignment Standards

Previous CBP studies have used program-generated levels of confidence as guidelines for accepting assignments. Researchers designate a confidence level cutoff (generally an 80% or 95% calculated confidence level) and accept all assignments made above the cutoff, and either consider assignments made below the confidence level as parasitic offspring from indeterminate parasite mothers or simply do not include them in final maternity assessments (Anderholm *et al.* 2009; Kreisinger *et al.* 2010; Tiedemann *et al.* 2011; Bird *et al.* 2013). We sought to evaluate if low confidence genetic assignments were less frequently supported by RFID evidence than high confidence assignments. COLONY calculates a probability between 0 and 1 for each assignment it makes. We binned assignments by probability in increments of 0.1 and assessed percent of assignments with RFID support across the spectrum of confidence level assignments.

### 2.2.7. Statistical Analysis

We conducted all statistical analyses using R (Version 3.5.1) in R Studio (Version 1.1.463). We performed linear models using the base R package to assess the relationship between the number of genetically detected parasitic offspring and 1) clutch size, 2) number of genetically detected females in a nest, 3) the number of RFID detected females in a nest, and 4) maximum egg accumulation rate recorded for a nest.

## 2.3. RESULTS

### 2.3.1. Population Level Detection of CBP

We genetically assigned 1194 offspring from the 103 nest attempts we sampled in 2016 (23 nests were not sampled). Genetic estimates of the population frequency of CBP were generally higher than the non-genetic estimates. The overall frequency of CBP detected varied from 19% to 92% based on the method used to detect parasitism (Table 2.1).

Genetics determined that 82% (n=84) of nests were parasitized in our populations (Table 2.1). While nests were parasitized at every clutch size, the proportion of parasite nests increased with increasing clutch size (Figure 2.1a). In addition, the intensity of parasitism—number of parasitic offspring per nest—increased with clutch size (Figure 2.1b, $r^2 = 0.45$, $p << 0.001$). The number of genetically determined parasitic offspring in a nest was strongly correlated with the number of females assigned offspring in the nest (Figure 2.2, $r^2 = 0.53$, $p << 0.001$). Genetic assignment methods indicated that 37% (n= 439) of the eggs laid in the three populations were parasitic (Appendix A2.2).

Compared to the genetic estimate of CBP, frequency of CBP from egg accumulation rates were low (Figure 2.3a). Egg accumulation estimated that 53% (n=55) of nests were parasitized across our study sites (Table 2.1). The number of genetically assigned parasitic offspring in a nest increased with an increasing maximum egg accumulation rate (Figure 2.3b, $r^2 = 0.13$, $p = 0.002$). Evidence from genetics and egg accumulation both concluded that 47% of the total nests were

parasitized (n=48). In the remaining 6% (n=7) of the nests that egg accumulation evidence detected parasitism, genetic methods did not.

The frequency of CBP detected using clutch size cutoffs varied widely depending on the clutch size used to determine parasitism (Table 2.1). The estimated frequency of CBP was highest with a maximum clutch size cutoff of 10 or more eggs: 92% of these nests (n=95) were parasitized. The frequency of nests assigned as parasitized declined steadily as the cutoff clutch size used to designate parasitism increased. In contrast, parasitism estimates based on RFID and/or genetics across clutch sizes reveal that nests of any clutch size may be parasitized (Figures 2.1, 2.4). In addition, although the parasitism rate for smaller clutch sizes is somewhat lower, parasitism is so prevalent that using a cut-off does not appear to be a very informative method of estimating CBP.

In terms of population frequencies, RFID estimates of CBP were most similar to genetic estimates, with RFID visits alone estimating that up to 83% (n=86) of nests were possibly parasitized (Table 2.1). Both RFID and genetic evidence found that 75% of nests (n= 77) were parasitized and 7% (n=7) were not parasitized (Figure 2.4). In 9% of the nests (n=9), RFID evidence indicated parasitism was possible, and genetic evidence did not identify any parasitic offspring. The remaining 5% of nests (n=5) were cases where genetic evidence indicated parasitism, but RFID evidence did not. In over half of these cases (n=3), genetic evidence indicated that all parasites were unsampled and untagged females, consistent with the failure of RFID to detect those cases of parasitism. RFID evidence is more likely to detect parasitism without

genetic support in smaller (below 15 egg) clutch sizes. Both RFID and genetic evidence detect parasitism more consistently as clutch size increases.

The mothers of 58% (n=254) of genetically assigned parasitic offspring were detected using RFID on the parasitized nest within a week of when their parasitic egg would have appeared. The number of genetically determined parasitic offspring in a nest had a weakly positive relationship to the number of unique RFID tagged females that visited the nest over the course of the attempt (Figure 2.5, $r^2 = 0.10$, $p = 0.001$).

### 2.3.2. Individual Tactic Determination and Fecundities

Genetic evidence alone determined that there were 110 actively breeding females in 2016. However, RFID evidence could only provide information on the activity of 77% of the females (n=85) assigned as mothers in COLONY outputs, as the remaining 23% (n=25) were females that results from COLONY indicated we had not genetically sampled and so were not RFID tagged in 2016. To make equal comparisons between genetically determined tactics and tactics determined by RFID and genetic evidence, we reduced our list of genetically assigned females to only include RFID tagged females. All of the untagged and unsampled females identified by COLONY only laid parasitically, so incorporating RFID evidence inherently resulted in an underestimate of parasite-only tactics.

RFID evidence largely supported the genetically determined tactics of the RFID tagged and genetically assigned females (Figure 2.6). With untagged females included, genetic evidence indicated that 20% (n=22) of females in our sample are

non-nesting parasites, which was reduced to 6% (n=5) when untagged females were removed. When all females were included, genetic evidence indicated that 47% of females are nesting parasites (n=52), and 33% (n=36) are non-parasitic females (Appendix A2.3). Without untagged females, genetic evidence indicated 56% of females are nesting parasites (n=34), and 38% (n=46) are non-parasitic females. RFID evidence confirmed the tactics determined by genetics for 84% (n=71) of the RFID tagged females. RFID evidence was also able to confirm that the female was scanned on the nest and available to lay the egg for every offspring she was assigned genetically for 67% (n=57) of these females. In all cases where RFID evidence did not support the genetically assigned tactic, nesting parasites that were converted into non-parasitic nesting females (n=14, 16%), as the only offspring RFID could not confirm for these females were parasitic offspring (Figure 2.7a).

While RFID evidence could provide support for individual tactics, it could not always confirm that a female was present to produce all offspring assigned to her, which reduced her overall fecundity. For 16% (n=14) of the remaining females, RFID evidence supported the genetically determined tactic but could not confirm all offspring assigned to the female. As a result, the female's effective clutch size was reduced by at least one and as many as 7 eggs (Figure 2.7b). Offspring could not be confirmed by RFID largely due to limitations of the method or our employment of it: 1) the female lacked an RFID tag when the egg was laid, but was tagged later in the season (3 females), 2) the RFID system itself was not operational during the laying period (3 females), or 3) the offspring assigned to the female were trapped and tagged

outside of a box, so their original box and host mother is unknown (2 females). An additional 6 females were never scanned on the nest during the laying phase, indicating were not on the nest when the parasitic egg was laid or they lost their tag.

### 2.3.3. Genetic Assignment Standards

We used RFID assessment as a check on the reliability of the genetic assignments as the probability of support calculated by COLONY varied. RFID evidence showed strong support for genetic assignments regardless of the calculated probability (Figure 2.8). RFID evidence was available for 83% (n=992) of the offspring in our populations. With a confidence level cut-off of 0.8, RFID evidence supported COLONY's choice of mother in 94% (n=648) of high confidence assignments (n=687) compared to 90% (n=274) of low confidence assignments (n = 305). Consequently, COLONY's assigned confidence level does not necessarily indicate risk of error, as confirmed by RFID evidence.

## 2.4. DISCUSSION

### 2.4.1. Population Level Detection of CBP

We found that estimates of CBP ranged widely depending on the method of detection and, except for RFID based estimates, non-genetic methods of CBP detection largely underestimated rates of parasitism. As such, non-genetic methods have little to no use as tools to refine genetic estimates of parentage. The finding that non-genetic patterns of parentage do not closely match genetic patterns is consistent

with other studies that compared genetics to non-genetic methods of parentage assignment. In a study of Soay sheep (*Ovis aries*), Coltman et al (1999) determined that estimates of paternity derived from the number of ewes associated with a ram were inaccurate, in part due to young males mating but not associating with ewes they impregnated. As with the non-nesting females in our study, these young rams went undetected as fathers as determined by behavioral patterns of parentage. Pemberton et al (1990) found that although behavioral estimates accurately represented relative success of individual polygynous red deer (*Cervus elaphus*) males, the actual number of offspring assigned behaviorally vastly underestimate the reproductive success of some males while overestimating the reproductive success of others. In a study of multiple maternity in cattle egrets (*Bubulcus ibis*), Moralez-Silva and Del Lama (2019) found that relying on egg laying observations and rates resulted in both assigning genetically determined parasitic offspring to the host (false negative), and assigning host offspring to parasites (false positive). Relying on non-genetic methods, in addition to not representing frequencies of behaviors accurately, risks misrepresenting the reproductive effort and success of subsets of the population.

The consistent underestimation of population level parasitism by non-genetic methods, while not accurate for studying CBP, do give insights into parasitic behavior in wood ducks which highlight future directions for studies of CBP in waterfowl. The fact that egg accumulation rates underestimated parasitism could indicate that host females either 1) lay one egg every 36-48 hours, 2) skip days between laying eggs, or 3) lay eggs in the nests of other females as they themselves are parasitized. The

assumption that wood ducks lay one egg per 24 hours has not been extensively tested, but given that Leopold (1951) found an average laying rate of 0.96 eggs per day, there is some evidence the assumption is accurate. Leopold (1951) also found that females did occasionally skip a day laying eggs in their own nest, so that could have contributed to our underestimation using this technique. If females are leaving their nests unattended to lay eggs elsewhere, it raises a central question in CBP literature: what is the cost of hosting parasitic offspring? This issue has received much theoretical attention, particularly as it pertains to kin selection (Andersson 1984, 2001; Zink 2000). If accepting parasitic eggs in one's own nest is costly, the risk of leaving the nest unattended to parasitize another nest should be outweighed by the benefit of laying eggs parasitically in another nest. Alternatively, incubating parasitic eggs and tending to extra offspring post-hatch may not be very costly, so parasitic eggs may not factor into host female behavior. Our analysis of parasitism by clutch size also indicates that in our populations, unparasitized nests are rare, regardless of clutch size. The pervasiveness of parasitism at all clutch sizes suggests that parasitism may not be so costly for the host, or the costs are balanced with the benefits of laying parasitically. To truly understand the costs and benefits of parasitic eggs to hosts, particularly those that are nesting parasites, accurate assignments are essential.

The failure of egg accumulation rates to detect parasitism as frequently as genetics may also indicate that much parasitic egg laying occurs in synchrony with the host's laying cycle. High synchrony and matching with the host has been shown in other avian species (Brown & Brown 1988; Lyon & Everding 1996; Ahlund &

Andersson 2001; Lemons & Sedinger 2011) and has been hypothesized to increase the value of parasitic offspring as it increases hatch success for waterfowl (Andersson & Åhlund 2012). A benefit of combining RFID with genetics could be examining host-parasite synchrony in detail to determine if hatch success is affected by the timing of the parasite laying eggs in the host's nest, although consistent daily nest visits to determine abnormal egg accumulation and onset of incubation could be a low-tech and low-cost substitute to this approach.

The underestimation of parasitism as detected by egg accumulation may be in part due to our implementation of the method. Egg accumulation as a method for detecting CBP is only as good as the researcher visitation rate, as every day a nest is left unchecked is an additional day where parasitic activity might be missed. Additionally, it is more difficult to determine the exact onset of host laying and incubation using this method without daily checks, which means post-laying (and even pre-laying) parasitism might go undetected (Frederick & Shields 1986). We did not check nests daily, and some went up to a week without a visit, so we undoubtedly missed some cases of parasitism, resulting in an underestimate of the behavior in part due to our nest checking schedule.

Lastly, the discrepancy between egg accumulation and genetic estimates of CBP could be due in part to sampling. We could not completely sample roughly half of the nests that egg accumulation indicated were parasitized but genetics did not (n=4), so genetics may not have detected the parasitic offspring laid during the incubation period. We did completely sample the other half of the nests (n=3), but in

these instances the laying period may have been longer than our estimate, and so these offspring might have been laid during the incubation period and therefore might not be parasitic.

The variability of abnormal clutch sizes to detect parasitism is in part an indication of the prevalence of parasitism in our populations and may hint at how parasites behave in our populations. Given the high frequency of parasitism in our populations, it is not surprising that abnormal clutch sizes were not an informative measure – nests of all clutch sizes had at least one parasitic egg in them. The number of eggs a parasite lays in a host nest, as well as how many nests she lays parasitically in, may influence the success of using clutch size cutoffs. Previous studies have shown that parasites are not uniform in terms of the number of parasitic eggs they lay or where they deposit them (Lyon 1993b; Jaatinen *et al.* 2011; Pöysä *et al.* 2014) and it appears parasites in our populations are also employing diverse parasitic laying patterns. If parasites in our populations were laying large quantities of parasitic eggs or all targeting a small subset of nests, abnormal clutch size might have been a better indication of parasitism.

RFID evidence estimated roughly the same proportion of parasitic nests in the population as genetic estimates did, although the nests RFID evidence indicated were parasitized did not align entirely with the nests that genetic assignments determined were parasitized. When RFID and genetic evidence did not agree on parasitism in a nest, it was more common for RFID evidence to indicate parasitism without genetic support for parasitism. This discrepancy could be partially explained by the fact that

we were not able to genetically sample all offspring in a nest and so may have missed detecting some parasitic offspring genetically. However, this pattern was present even in nests where all offspring were sampled, and so females appear to be visiting nests for purposes other than egg laying. Previous studies indicate that wood ducks prospect nests before deciding to lay eggs, although it is unclear what information they may be gathering during these visits (Bellrose & Holm 1994). Future studies should be able to shed light onto prospecting and other non-laying visits to nests by using the strength of patterns of genetic maternity to elucidate behavioral visitation patterns based on RFID activity.

While genetic and RFID evidence of CBP were largely concordant at the population level, there were instances where genetic methods were able to detect parasitism when RFID methods were not, which lends support to the strength of genetic assignment techniques. Genetic methods detected parasitism at 5% of nests (n=5) that RFID evidence indicated were not parasitized. This discrepancy can, at least in part, be attributed to a potential weakness of RFID: it cannot detect female visits if the female is not tagged, while genetics can infer the genotype of an unsampled female and assign offspring to her. Even if we were to accept those nests as indications of genetic error instead of RFID error, they do not alter the results of our population level estimates of parasitism much, as parasitism was widespread even when we restricted our analyses to nests that RFID and genetic evidence agreed upon. Frequency of CBP varies widely across species (Yom-Tov 2001), so the acceptable level of error in detection should be proportional to the frequency detected. If

parasitism is relatively abundant, as in our study, an error rate of 5% or more does not change the frequency of the behavior in the same way that it would in a population with relatively low detected levels of parasitism. Some studies have discovered low rates of CBP (under 5%) and should be take extra measures to ensure that they are detecting a relatively rare behavior rather than one that does not exist in the population.

The inaccuracy of previous studies of CBP that were entirely dependent on non-genetic methods of parentage assignment is hard to assess, as these studies do not have genetic information associated with them, and any corroboration was done with other non-genetic information. For example, while using evidence from clutch size cutoffs generally resulted in an underestimate of parasitism in our study, another study of wood ducks found it overestimated CBP, although this conclusion was based on CBP estimates using egg accumulation rates (Semel & Sherman 2007). In contrast, Lyon and Everding (1996) found that clutch size evidence reliably detected parasitized nests in eared grebes (*Podiceps nigricollis*), as verified by egg accumulation evidence. Researchers should evaluate the merit of re-evaluating conclusions based on non-genetic estimates of behaviors using genetic estimates if possible, considering the risk that the behavior went under-detected in the past.

It should be noted that there are several ways to measure conspecific brood parasitism, and each different measure applies to different questions. Since selection works on individuals, population level estimates of CBP have limited applications in understanding the evolution of the behavior beyond comparing groups or taxa. In our

populations, parasitism was so pervasive at the population level that nest-level estimates cannot be extrapolated to estimating the frequency of parasite tactics. This is in line with previous studies that looked at population level estimates of parasitism and compared them to the proportion of parasite eggs in the population (Eadie *et al.* 2010).

### 2.4.2. Individual Tactic Determination and Fecundities

Individual tactics and fecundities determined by genetics alone were largely supported by RFID evidence, which further enforces the strength of genetic assignment as an approach to determine parentage. Instances where RFID and genetic evidence disagreed on assignments were relatively rare and could occasionally be explained due to sampling issues. However, as with genetics, RFID evidence also comes with its own inherent complications and biases, particularly when estimating parasite tactics and fecundities.

Non-nesting parasites are at the highest risk of not being detected, regardless of technique; because they do not nest, they mostly go uncaptured and thus both lack RFID tags and blood samples for genetic detection of CBP. However, because COLONY can infer genotypes of parents missing from a candidate parent pool, parasitic offspring of presumably unsampled non-nesting parasites can be assigned to putative mothers. Most of the assignments unsupported by RFID evidence were offspring assigned to unsampled inferred mothers, as RFID could not confirm assignments to females that were never sampled and thus never RFID tagged. RFID

evidence accurately assessed the occurrence and frequency of parasitism by nesting females but there were instances where parasite offspring produced by nesting parasites were not supported by RFID evidence, and inference from RFID alone would suggest that these were the host's offspring. If we were to eliminate genetic assignments not supported by RFID evidence, it is the parasitic categories that would be misrepresented the most, with many non-nesting parasites vanishing entirely from the candidate parent pool, reducing the perceived frequency of the tactic dramatically and limiting estimates of reproductive effort and success.

When working with RFID evidence in combination with genetic evidence of CBP, it is important to consider the risks and rewards associated with relying too much on non-genetic sources of information. Genetic methods are strong on their own and relying on them appears to be a relatively robust approach for parentage determination, given that RFID evidence concurs with genetic estimates of tactics and fecundities for the majority of our data. Our previous study indicates that with a good set of markers and thorough sampling, the risk of assigning a non-parasitic offspring to a parasite is extremely low, so overestimating parasitism is unlikely to be a factor in our study system. Incorporating RFID evidence comes with the risk of underestimating parasite tactics, but as long as the RFID system is reliable and all individuals are tagged, can provide incredibly rich additional behavioral context for genetic patterns of parentage. To obtain the richest understanding of patterns of parentage and reproductive behavior, we urge researchers to trust the genetics first,

and use RFID activity (and other non-genetic evidence) to explore patterns of breeding activities rather than as a tool to refine genetic assignment.

### 2.4.3. Genetic Assignment Standards

Variation in the COLONY assigned confidence level did not reflect variation in the reliability of the assignment as determined by RFID evidence. Lower probability assignments were just as frequently supported as higher probability assignments, suggesting that the probability as calculated by COLONY does not always indicate of risk of error. RFID evidence supported over 90% of assignments, regardless of the probability COLONY calculated for the assignment. Most of our assignments had calculated probabilities that were higher than the traditional confidence level cut-offs of 80% and 95%, but if we were to remove lower confidence assignments from our parentage assessments, we would have unnecessarily eliminated 23% (n=272) of the assignments made by genetics, primarily those assigned to parasitic females.

### 2.4.4. The Role of Non-Genetic Information

Although non-genetic parentage assignment methods do not appear to enhance genetic parentage assignment, genetic assignments can help shed light on patterns derived from non-genetic observation methods of reproductive behaviors. In conjunction with genetic assignments, future research can address nest prospecting

and visitation and potential interactions between hosts and known parasites using RFID activity. Egg accumulation rates in combination with genetics can address questions about parasite laying synchrony and host female laying rates and activity.

Additionally, genetic inference is often strong, but not infallible. For example, genetic assignments are prone to increased risk of error when fewer markers are included (Thow et al, manuscript), which increases the corroborative value of RFID and other remote sensing data. Without an external check, researchers risk interpreting patterns of parentage that are partially or entirely false and open their work to criticism, whether founded or not. However, with the advent of genomics, the number of markers included in analyses is less likely to be an issue, and so non-genetic information may lose corroborative value as these approaches become widespread.

While non-genetic information is not as robust as genetics in terms of determining patterns of parentage, it still has value in terms of providing important context for ARTS. Without additional information on the condition, experience, and breeding success of candidate parents, many hypotheses explaining ARTS are not truly testable as potential drivers of the behavior cannot be considered thoroughly enough to come to robust conclusions about causality (Griffith *et al.* 2002). In CBP in particular, the costs and benefits of the behavior are essential for understanding if the behavior is truly parasitism or a case of cryptic cooperation (Zink 2000; Andersson 2001), and both genetically determined patterns of parasitism as well as

ecological information on candidate parent behavior and reproductive success are fundamental to calculate those parameters (McRae & Burke 1996; Dickinson 2007).

Molecular methods of parentage assignment are strong enough to be relied upon alone for determining patterns of parentage in ARTS, but it is important to consider multiple lines of evidence in order to draw the most robust conclusions about the overall evolution and dynamics of a behavior. A holistic approach towards understanding ARTS is critical, as the ecological context of a behavior is as important as finding out which individuals are responsible for it in order to understand how these behaviors evolve and are maintained.

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**Table 2.1**. A summary of the frequency of parasitized wood duck nests (total = 103 nests) using one line of evidence alone or two combined lines of evidence to identify parasitized nests. We assigned nests with insufficient evidence to the Undetermined category. When we used two lines of evidence resulted in one line of evidence determining the nest was parasitized and the other line determining the nest was not parasitized, we assigned the nest to the Conflicting Evidence category. See Appendix A2.1 for site-specific information.

| **Method** | **Parasitized** | | **Not Parasitized** | | **Undetermined** | | **Conflicting Evidence** | |
|---|---|---|---|---|---|---|---|---|
| | Nests | Percent Total | Nests | Percent Total | Nests | Percent Total | Nests | Percent Total |
| **Single Line of Evidence** | | | | | | | | |
| Genetics | 84 | 82% | 19 | 18% | 0 | 0% | — | — |
| RFID | 86 | 83% | 12 | 12% | 5 | 5% | — | — |
| Egg Accumulation | 55 | 53% | 31 | 30% | 18 | 17% | — | — |
| Clutch Size Cutoffs | | | | | | | | |
| 10 | 95 | 92% | 8 | 8% | 0 | 0% | — | — |
| 12 | 88 | 85% | 15 | 15% | 0 | 0% | — | — |
| 14 | 72 | 70% | 31 | 30% | 0 | 0% | — | — |
| 16 | 51 | 49% | 52 | 50% | 0 | 0% | — | — |
| 18 | 36 | 35% | 67 | 65% | 0 | 0% | — | — |
| 20 | 29 | 28% | 74 | 72% | 0 | 0% | — | — |
| 22 | 20 | 19% | 83 | 81% | 0 | 0% | — | — |
| | | | | | | | | |
| **Two Lines of Evidence** | | | | | | | | |
| RFID and EA | 47 | 46% | 8 | 8% | 17 | 17% | 26 | 25% |
| RFID and Genetics | 77 | 75% | 7 | 7% | 5 | 4% | 14 | 14% |
| Genetics and EA | 48 | 47% | 12 | 12% | 18 | 17% | 25 | 24% |

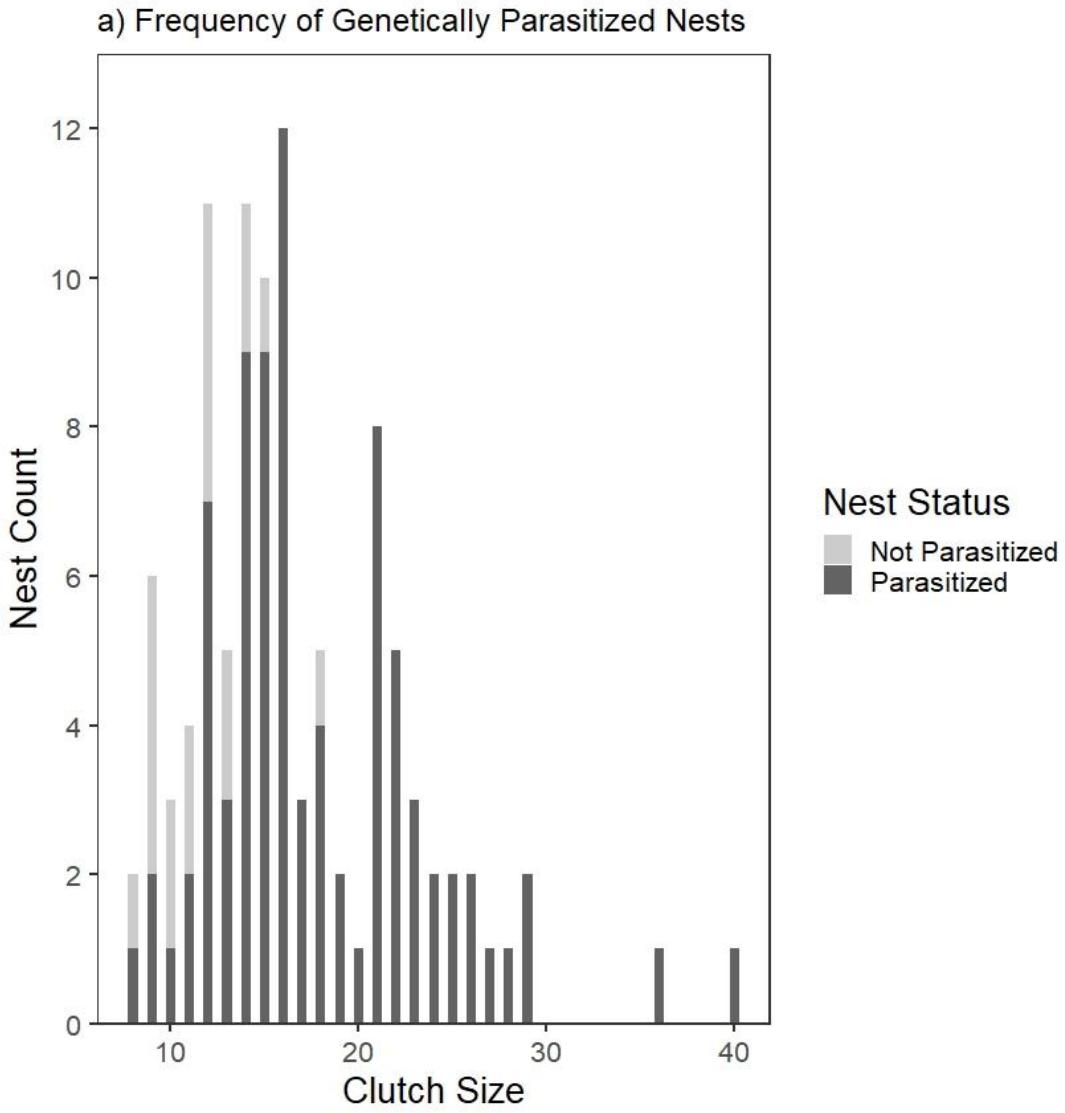


**Figure 2.1.A.** The frequency of CBP at the population level as determined by genetic methods (n=103 nests). If a nest had one (or more) offspring that was not assigned to the host mother for that nesting attempt, the nest was considered parasitized.

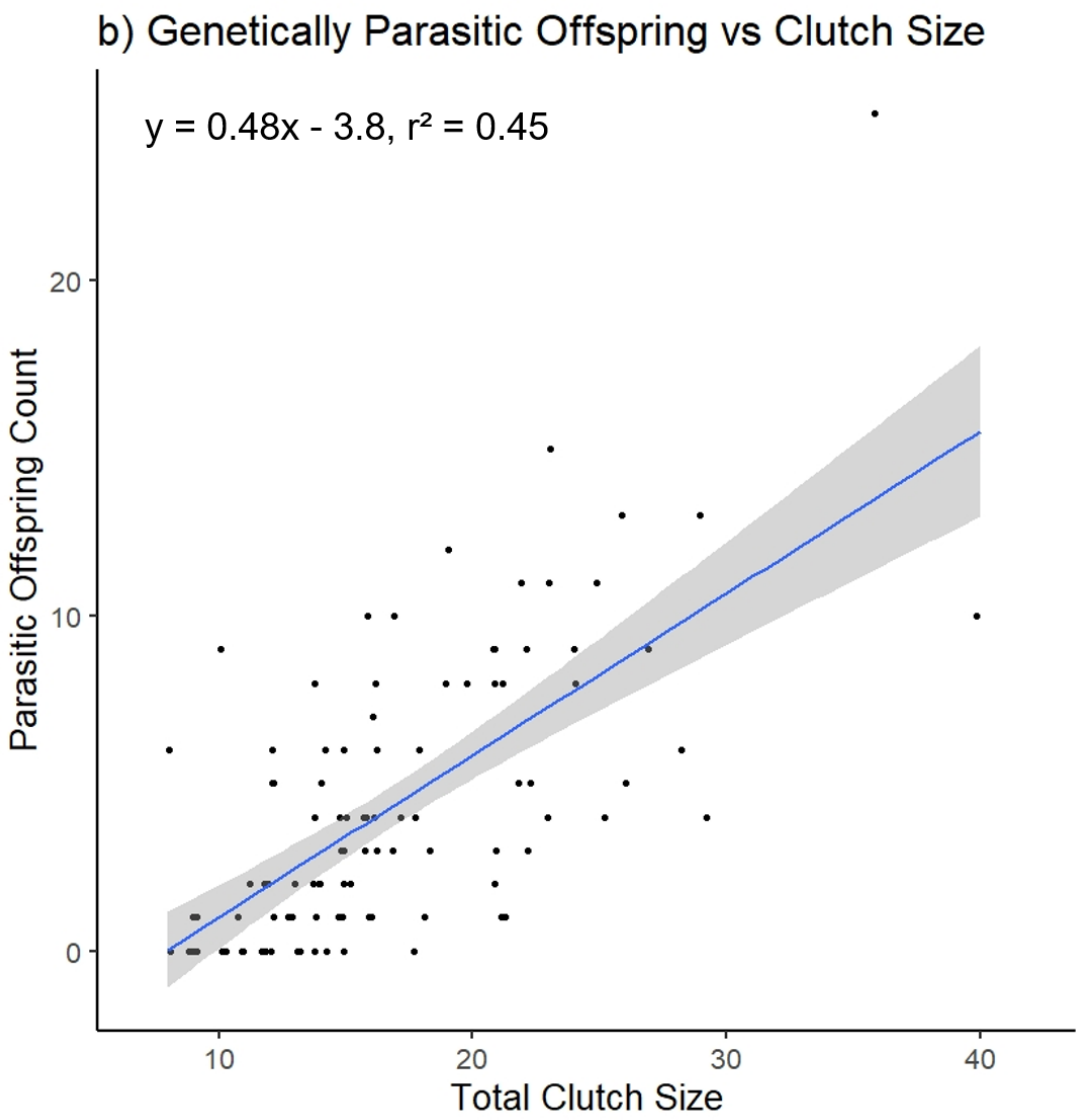


**Figure 2.1.B.** The relationship between clutch size and the number of parasitic offspring as determined by genetic methods (n=103 nests). The grey area indicates the 95% confidence interval.

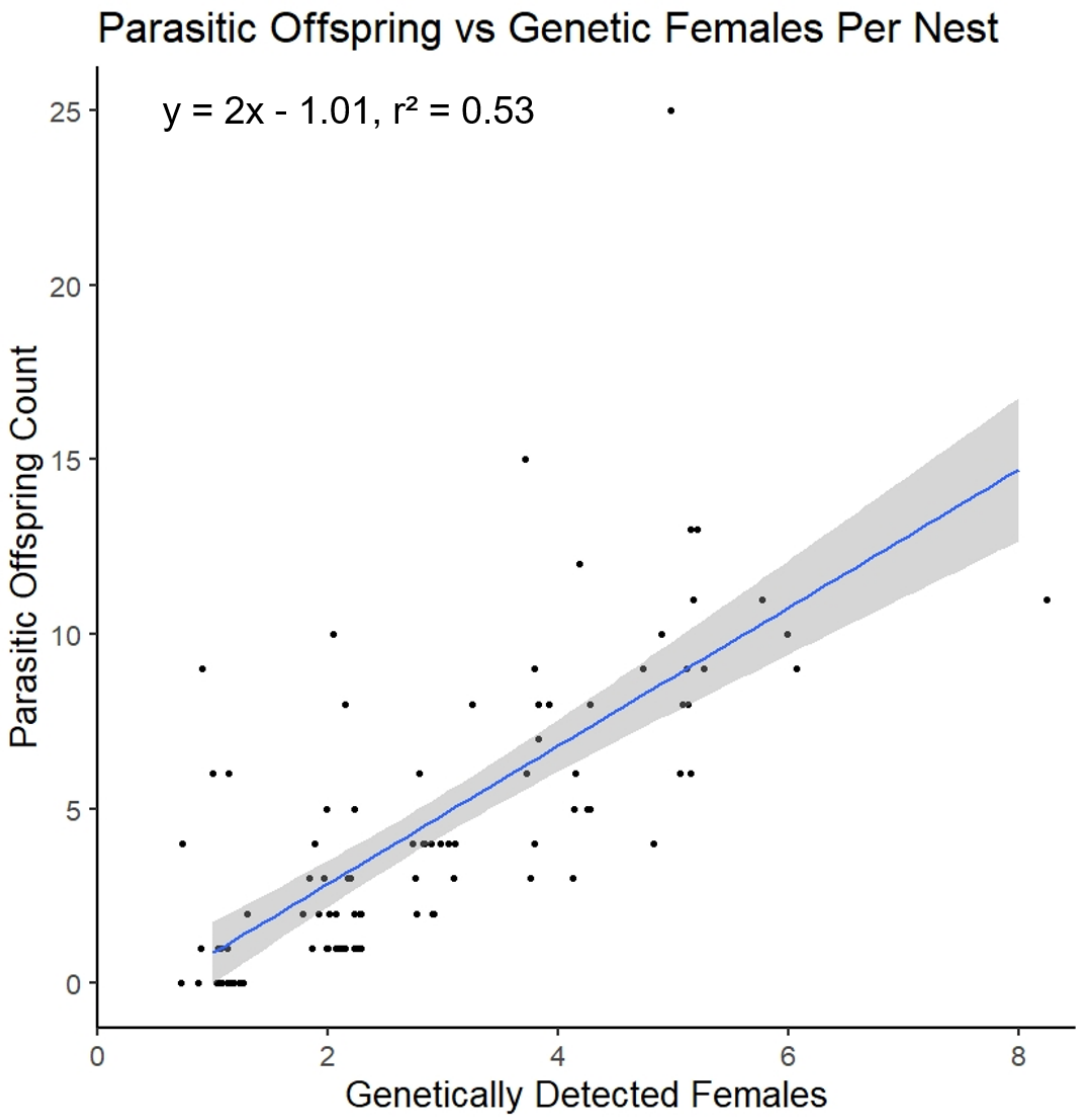


**Figure 2.2.** The relationship between the number of females assigned offspring in given nest and the total number of parasite offspring in the nest. Parentage was determined with genetics for 103 nesting attempts. The grey area indicates the 95% confidence interval.

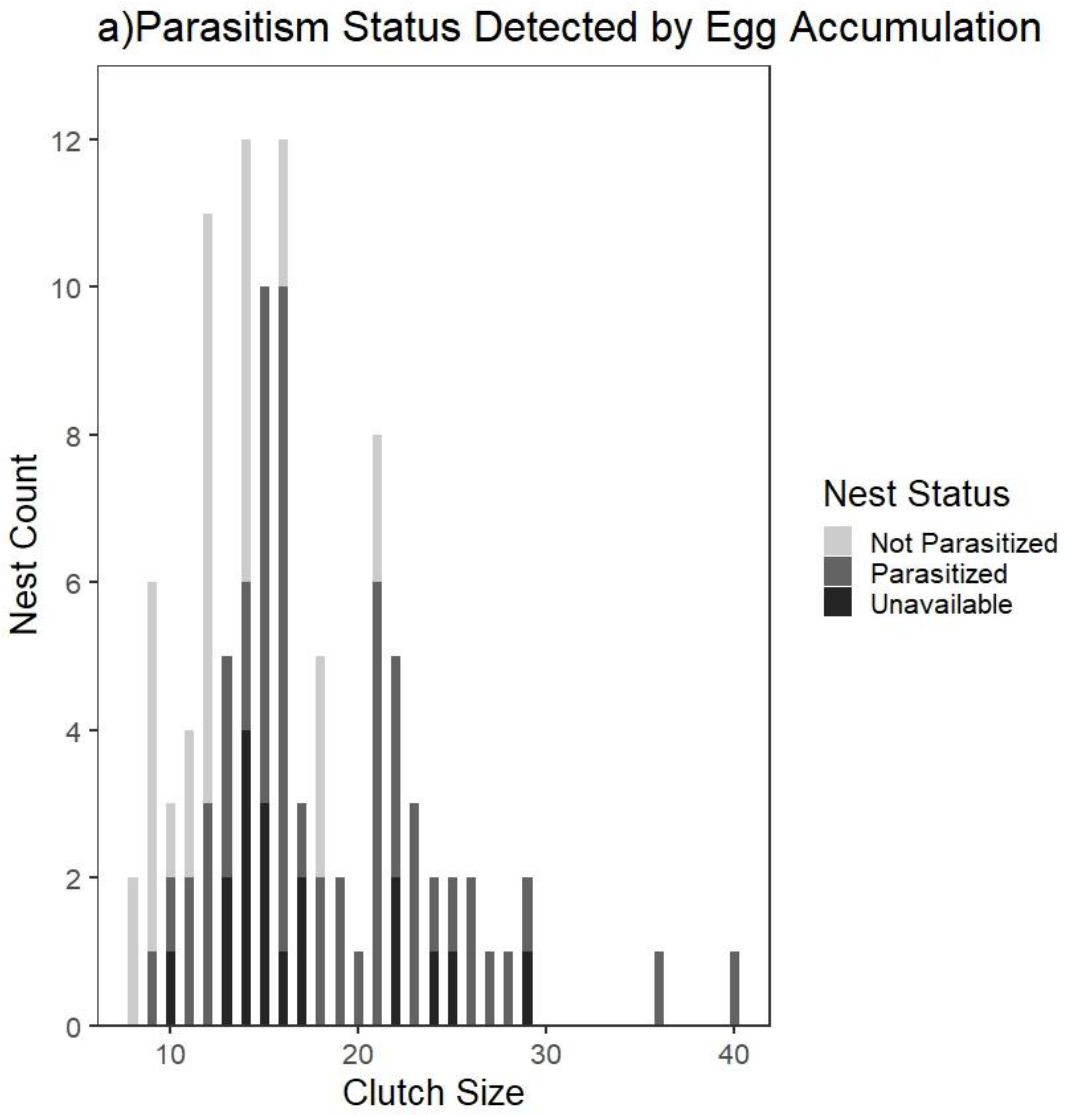


**Figure 2.3.A.** CBP detected by abnormal egg accumulation rate as measured by population level frequency (n=103). We considered a nest parasitized if at any point more than one egg appeared in a 24-hour period.

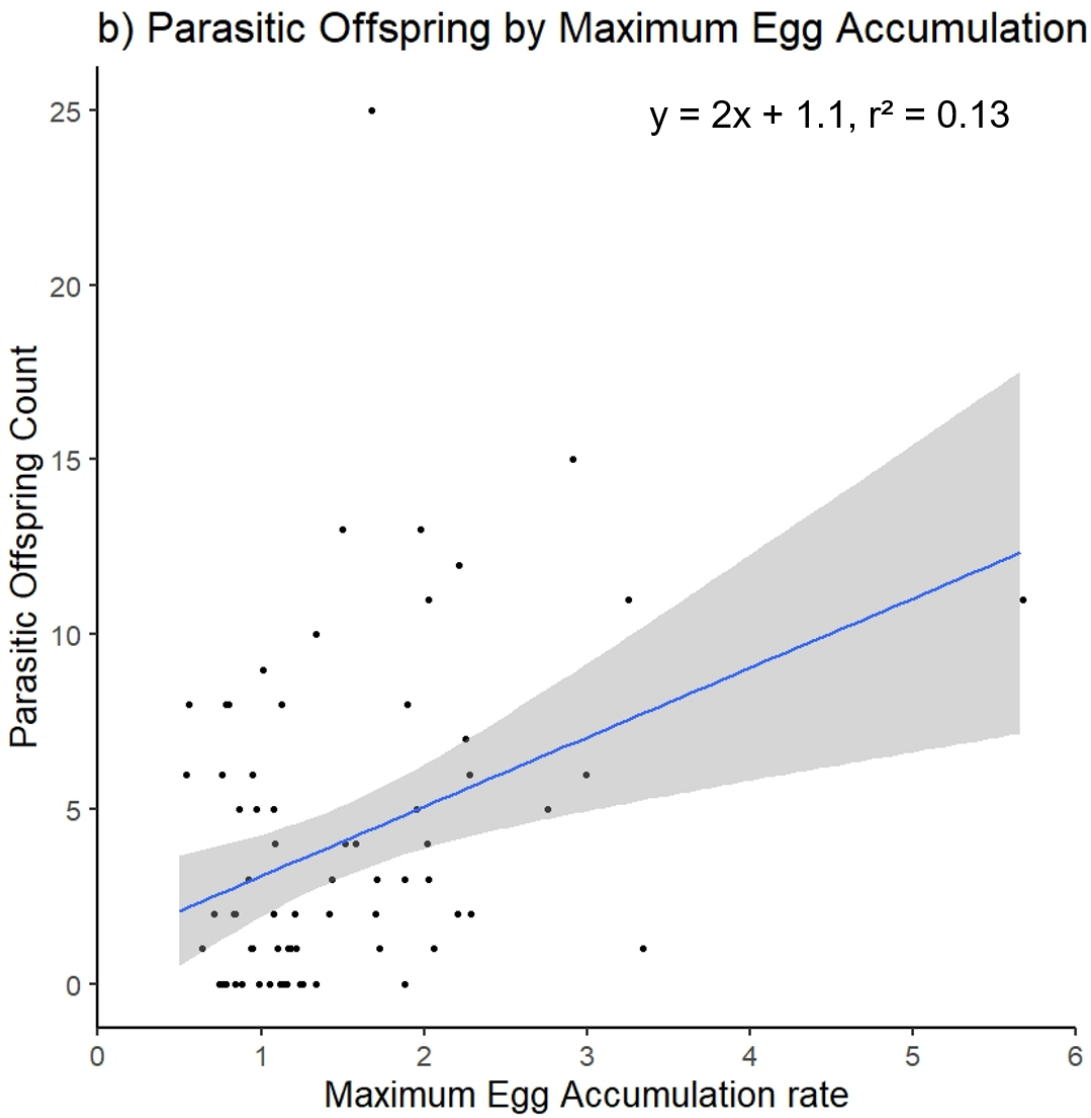


**Figure 2.3.B.** CBP detected by abnormal egg accumulation rate as measured by parasitism intensity (n=72). We compared the number of genetically determined parasitic offspring found in a nest to the maximum number of eggs to appear in a nest in a 24-hour period. The grey area indicates the 95% confidence interval.

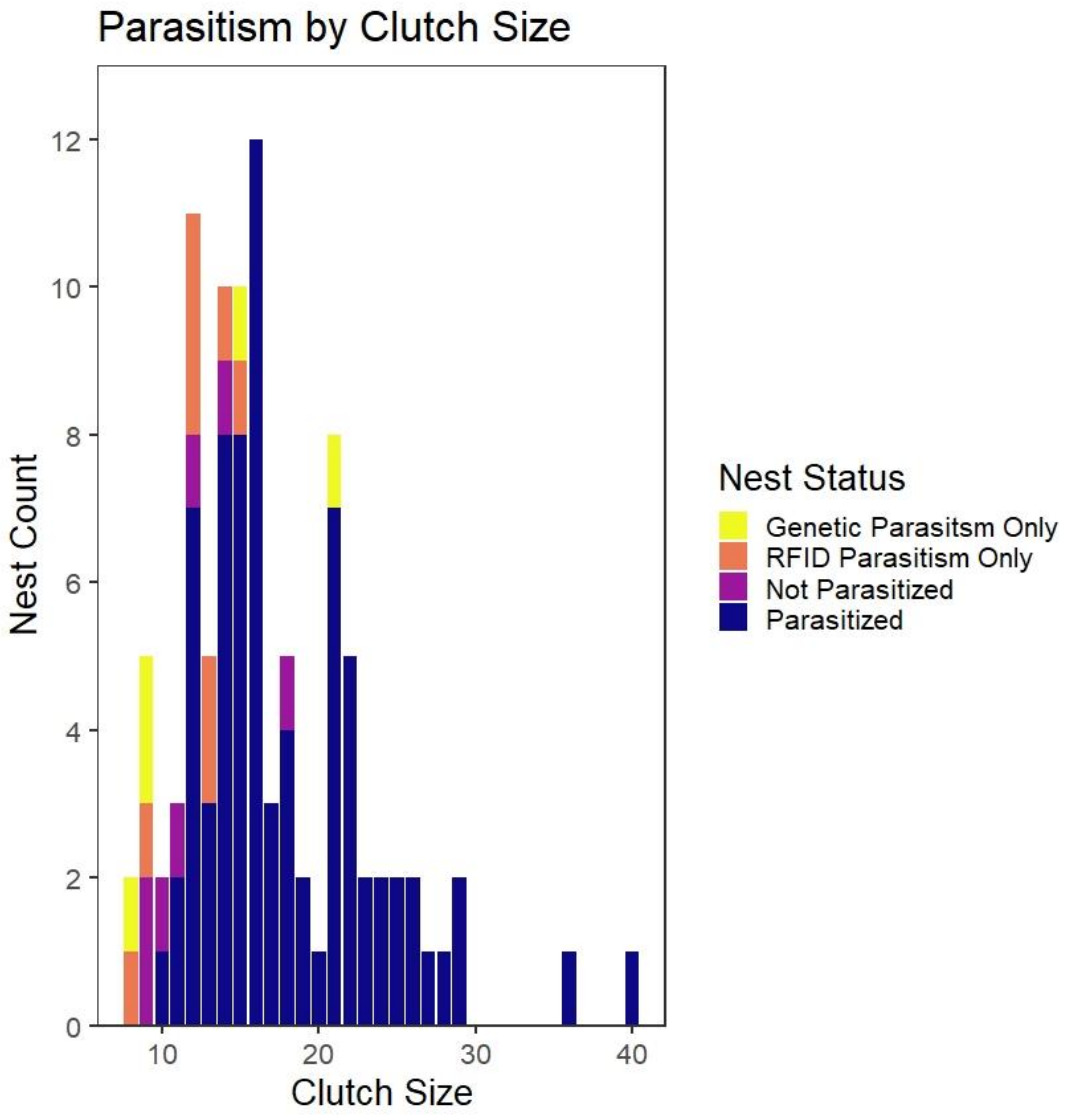


**Figure 2.4.** CBP as determined by RFID reads and genetics in comparison to the total number of eggs counted in the nest for 98 nest attempts across 3 sites in Yolo County, CA. While both methods came to the same conclusion in most cases (Parasitized and Not Parasitized categories), there were cases where RFID detected parasitism and genetics did not (RFID Parasitism Only) or genetics detected parasitism and RFID did not (Genetic Parasitism Only).

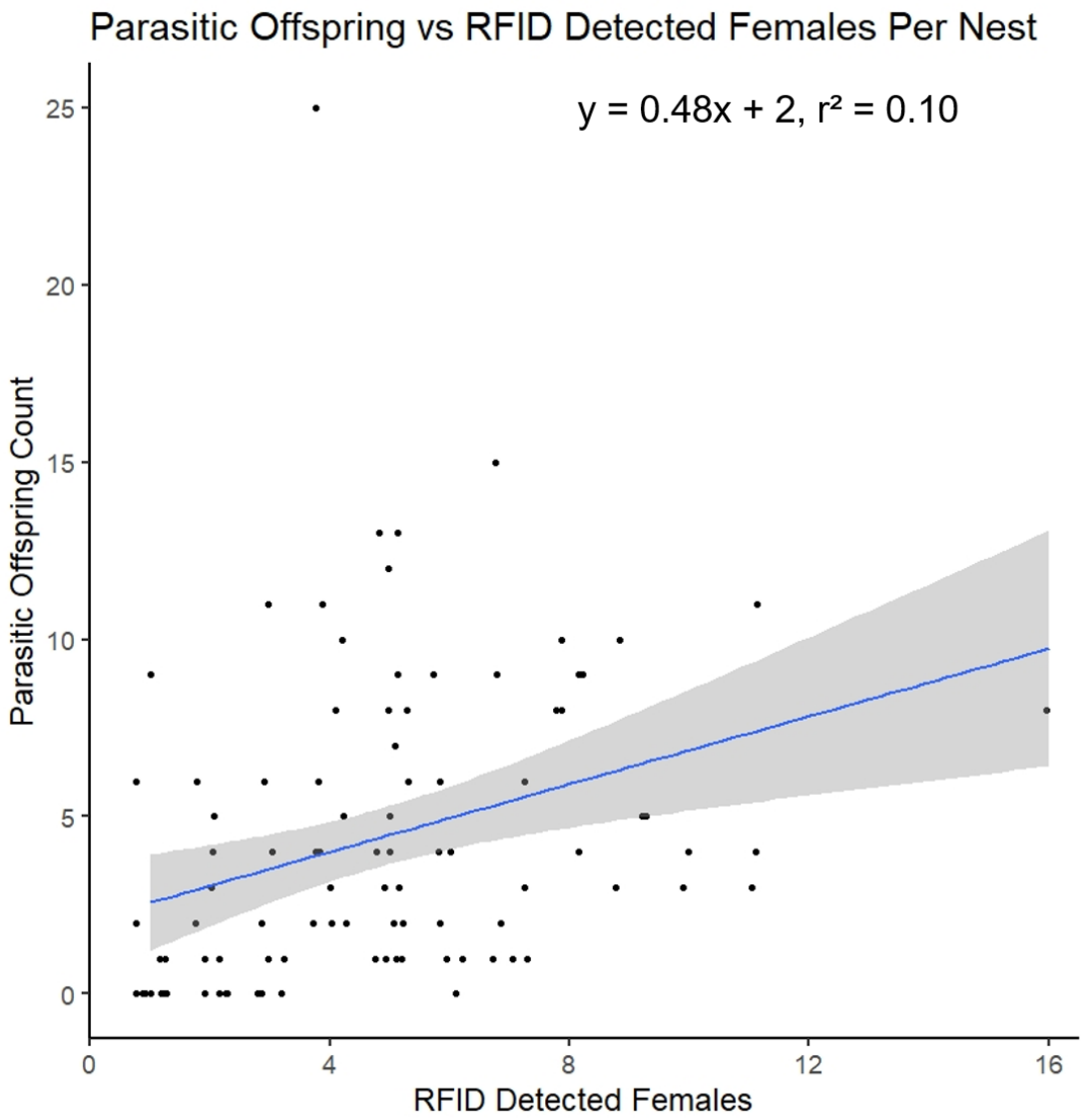


**Figure 2.5.** The intensity of parasitism (as measured by the number of parasite offspring determined by genetics) compared to the total number of unique females visiting each nest during a nest attempt (determined by RFID visits). The grey area indicates the 95% confidence interval.

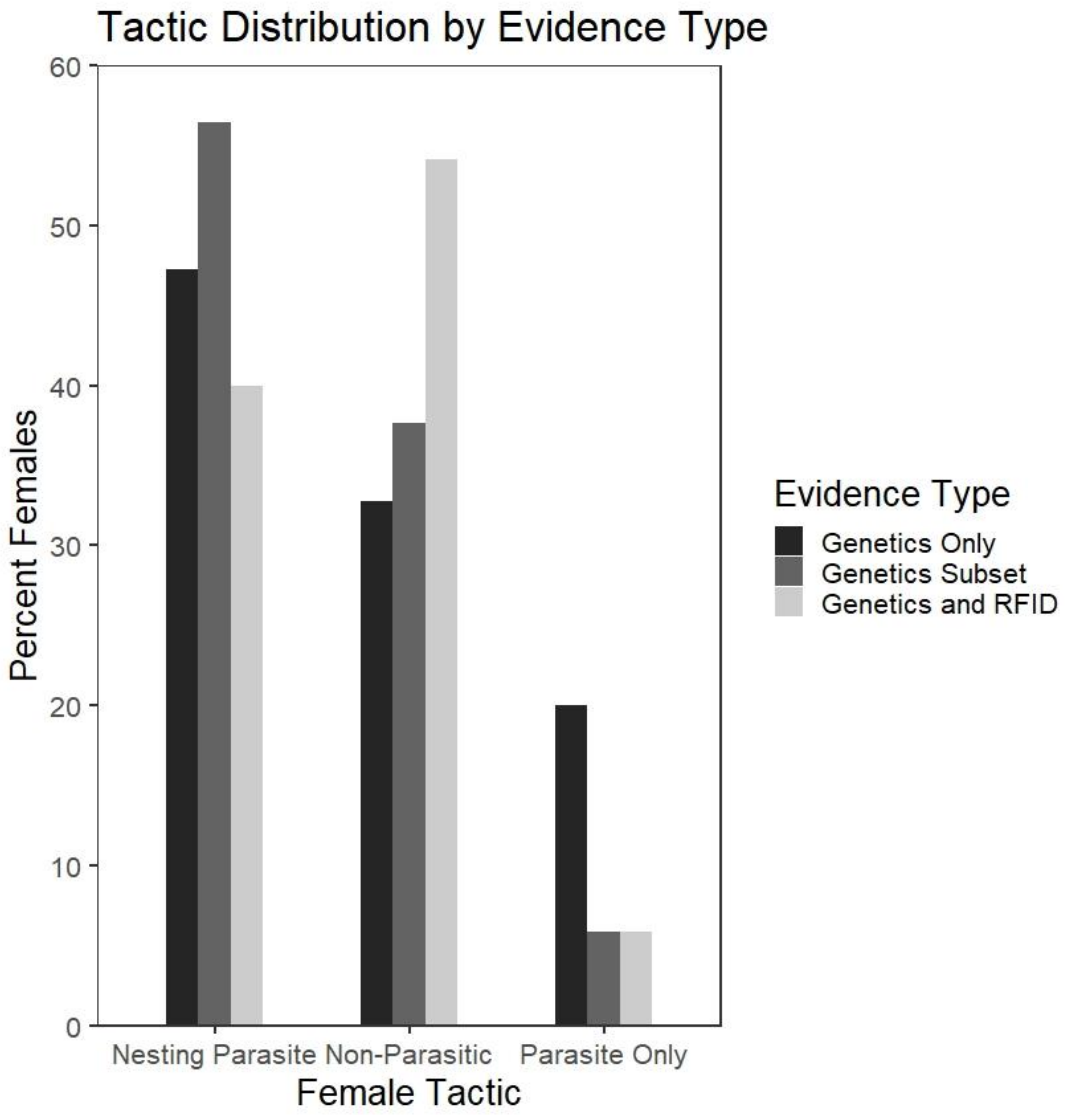


**Figure 2.6.** The distribution of CBP tactics as determined by genetic evidence alone and with RFID evidence incorporated into genetic evidence. Although COLONY assigned 110 females as mothers in the maternity analysis (Genetics Only), 25 of the females identified by genetics were either unsampled or otherwise not RFID tagged at the time their assigned offspring were produced. As a result, only 85 of the original 110 females were considered when RFID evidence was incorporated to corroborate genetically determined tactics. The females included in the genetic subset for RFID comparison (Genetics Subset) are the same 85 females in the Genetics and RFID category, the only difference being that RFID evidence is incorporated into the latter group for tactic determination.

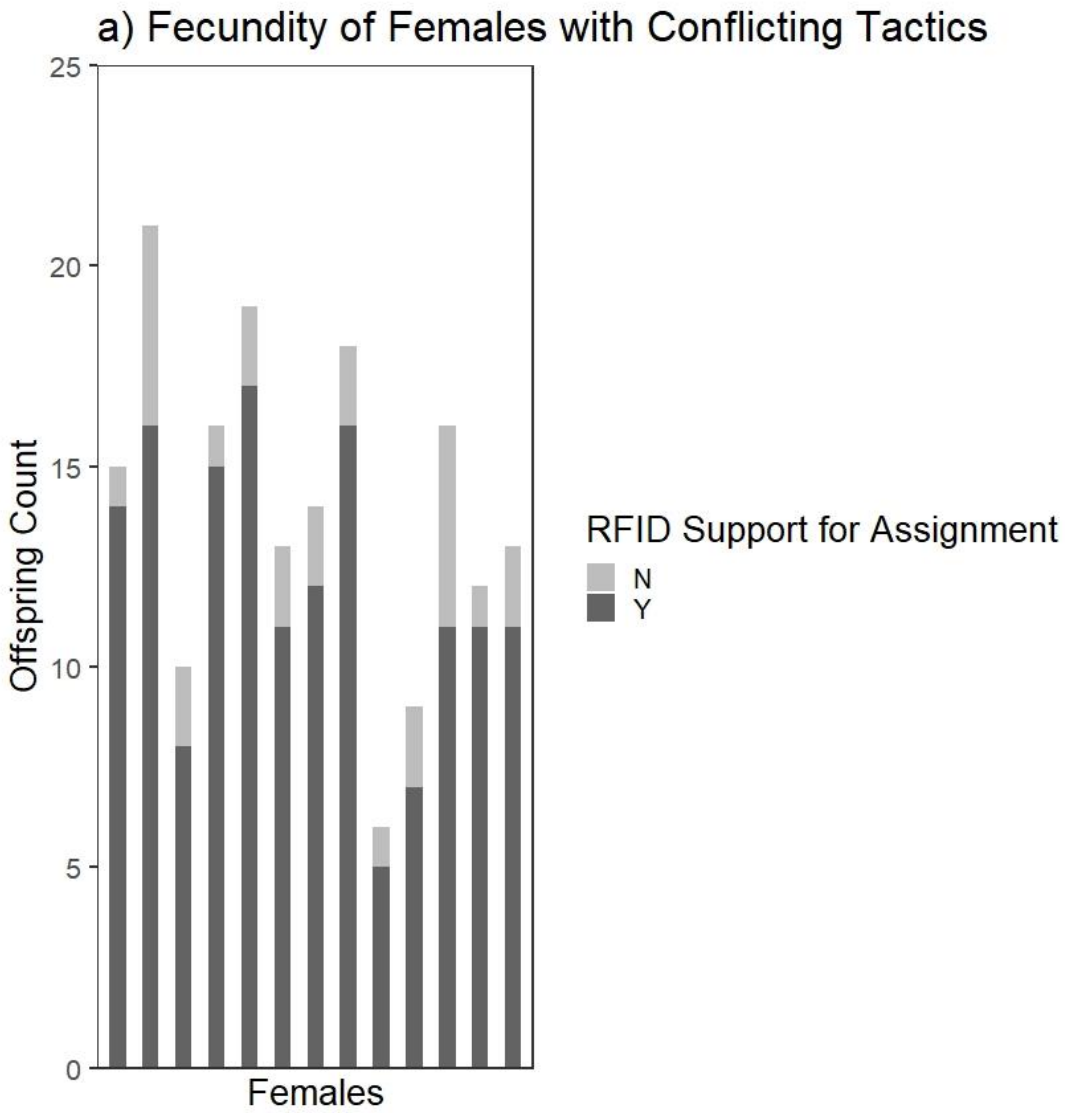


**Figure 2.7.A.** Differences in female fecundity based on RFID support for offspring assignment in females where RFID and genetic evidence pointed to different tactics (n=14). All females where RFID and genetic evidence conflicted on tactic assignment were genetically assigned as nesting parasites, but RFID could not confirm their parasitic offspring, so they were converted to non-parasitic nesting females.

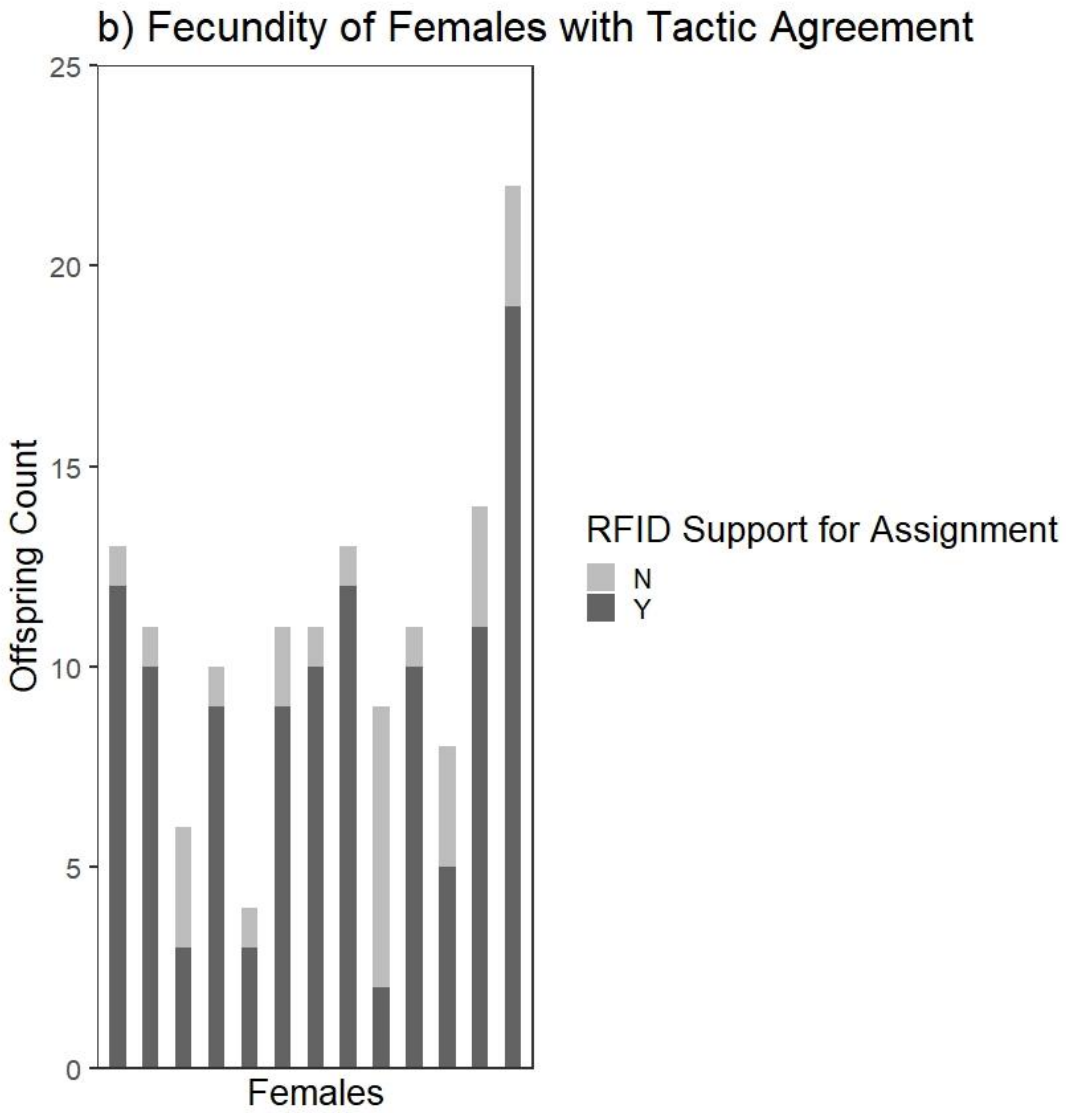


**Figure 2.7.B.** Differences in female fecundity based on RFID support for offspring assignment in females where RFID and genetic evidence agreed on tactic assignment (n = 13). Of the females where RFID and genetics agreed on tactic, 11 were nesting parasites and 2 were females that only parasitized.

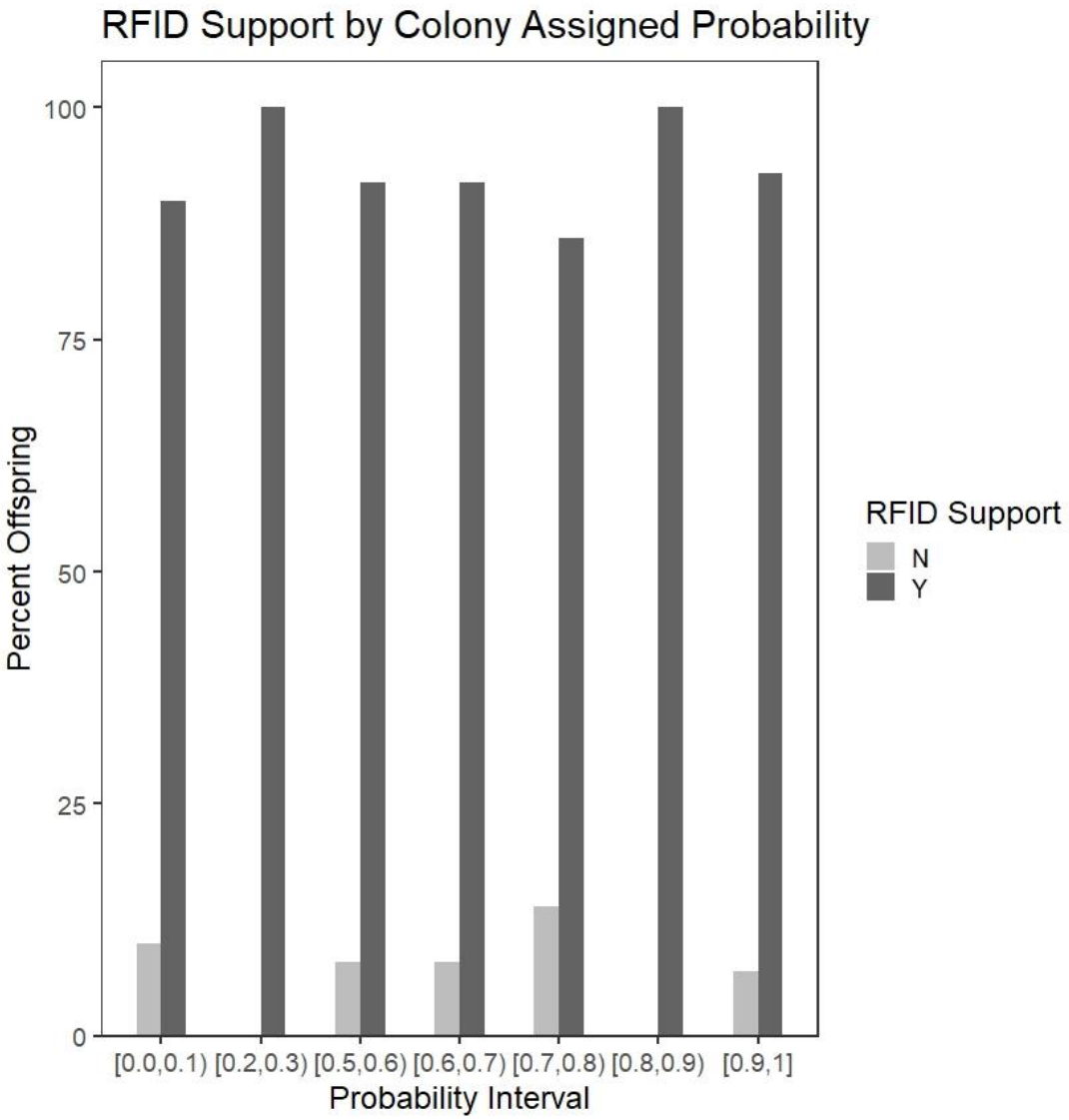


**Figure 2.8.** Genetic assignments (n=991) sorted by the probability calculated by COLONY for the assignment and whether RFID activity indicated the assigned mother was available to produce an offspring. COLONY assigns a probability of between 0 and 1 for each assignment it makes. We calculated the percentage of assignments in each probability interval that were and were not supported by RFID evidence. RFID support was determined by the presence of COLONY's choice of female on the nest during the time period her assigned would have been deposited as an egg. Missing probability intervals indicate there were no assignments made in that probability interval.

# CHAPTER 3: Tactics and transitions: the architecture of alternative reproductive options in wood ducks (*Aix sponsa*)

## 3.1. INTRODUCTION

The maintenance of behavioral variation is a key question in evolutionary biology, particularly with respect to behaviors that have strong fitness consequences. Alternative reproductive tactics (ARTs) are suites of discrete reproductive behaviors that directly affect fitness, and therefore should be under strong selection (Taborsky *et al.* 2008). Although the literature is divided over naming convention between strategies and tactics, we will follow the convention set forth by Taborsky, Oliveira, and Brockmann (2008) by using the term ARTs to refer to the combined set of behaviors, and refer to each behavior within the ART as a reproductive option. Alternative reproductive tactics are generally considered either as sets of multiple, fixed behavioral phenotypes (fixed ARTs) or a single, plastic phenotype (flexible ARTs) that allow individuals to adopt multiple reproductive options over the course of their lifetimes (Gross 1996; Taborsky *et al.* 2008). Fixed ARTs are relatively well understood mechanistically at many levels, due to rigorous and comprehensive long-term studies on model systems (Morris *et al.* 1992; Lank *et al.* 1995; Sinervo & Lively 1996). Flexible ARTs have been harder to pin down, possibly due to the diversity of situations in which they are expressed and lack of consistent long-term studies.

Previous literature has focused on categorizing flexible ARTs as mixed or conditional (Plaistow *et al.* 2004). However, this approach to categorizing ARTs appears limited; mixed ARTs are thought to be composed of reproductive options that result in equal fitness, and thus are adopted probabilistically, but no clear example of this exists in nature (Gross 1996). Conditional ARTs are defined as reproductive options that optimize fitness based on any number of prevailing conditions an individual may be experiencing in a breeding season; thus, almost every ART can be described as conditional (West-Eberhard 1987; Taborsky & Brockmann 2010) and the distinction loses power. Other approaches to classifying ARTs have been developed (Caro & Bateson 1986; Neff & Svensson 2013) but some recent studies still apply the mixed versus conditional approach (Schradin & Lindholm 2011; Hill *et al.* 2015).

To this point, ARTs have been quantified as single-season alternatives, with a heavy emphasis on correlates of reproductive success. This approach can isolate conditions that may be contributing to behavioral variation, reproductive success, and frequency of reproductive options in that season, but for iteroparous species, any conclusions we draw from these seasonal correlations may not fully explain potential lifetime patterns of reproductive success. Simply looking at a seasonal snapshot may lead to the wrong conclusions about which reproductive options have higher fitness (Taborsky 2008). Hypothetically, if a seasonal assessment determined that an individual adopting reproductive option A has lower reproductive success than one adopting reproductive option B, one might conclude that reproductive option A was

an example of a best-of-a-bad-job (BOBJ). However, if one were to track reproductive success of those two individuals for multiple seasons, they might find that the individual that initially adopted reproductive option A survived many more seasons than the individual that initially adopted reproductive option B, and ultimately achieved higher lifetime reproductive success as a consequence. In this scenario, the original conclusion that reproductive option A was an example of a BOBJ would be misleading about the lifetime fitness consequences of adopting that reproductive option.

In addition, many single season studies do not consider individuals that abstain from breeding for the season as they are not as easily tracked, although abstaining from breeding may be an important aspect of an individual's lifetime reproductive trajectory (Williams 1966; Curio 1983; Brown & Weatherhead 2004). Single season snapshot studies at best provide an incomplete assessment of reproductive options and their consequences, and at worst could be misleading. It is critical to identify all reproductive options available to individuals and consider how they are packaged over multiple breeding seasons (and ideally their lifetime) to fully understand the fitness consequences of ARTs.

Life history theory provides an alternative approach to understanding the evolution and maintenance of ARTs by framing reproductive option choice longitudinally. Taking a life history perspective, the reproductive options displayed over multiple seasons coalesce into a behavioral trajectory which determines lifetime fitness. A longitudinal view is also necessary to put into context how flexible

behaviors are (by examining transitions across breeding seasons), what correlates may influence reproductive option selection, and what the lifetime fitness consequences of behavioral trajectories are (Lyon & Eadie 2008). Considering reproductive options in the context of multiple seasons places the emphasis on trade-offs over the course of an individual's lifetime, rather than snapshots of conditions that individuals face in a single season.

Previous research has focused heavily on factors determining male ARTs (Henson & Warner 1997; Neff & Svensson 2013), but although the flexibility of female ARTs like CBP have been demonstrated many times (Eadie 1989; Forslund & Larsson 1995; Ahlund & Andersson 2001; Lyon & Eadie 2008; Hill *et al.* 2015) they have received less attention. Reproductive options may be variable throughout time due to female age, individual quality, early life experience, personality, or ecological factors like population density and/or breeding site availability. These factors combine to determine resources available for breeding in each season and how they are acquired, and so influence the costs and benefits of adopting reproductive options. The reproductive options females adopt translate to population level trends and dynamics, which in turn influence our understanding of how reproductive options evolve and are maintained in populations with female ARTs.

Conspecific brood parasitism is a female ART that occurs when a female contributes at least one egg to a nest that she does not incubate, relying entirely on another female to care for her offspring. Parasitic females may or may not have their own nest in addition to the eggs they lay parasitically. Studies of CBP would benefit

from a life history perspective, as CBP females are generally iteroparous and CBP females can display one of four reproductive options: 1) abstain entirely from breeding for a season, 2) only lay parasitically, 3) nest without parasitizing another nest, or 4) nest and parasitize other nests (Lyon & Eadie 2018). Previous research in waterfowl has suggested these reproduction options scale with condition or experience (Sorenson 1991; Lyon & Eadie 2008). Certain reproductive options may be more likely as females age or obtain more experience, as has been observed in male ARTs (Caro & Bateson 1986; Taborsky *et al.* 2008), resulting in predictable trajectories due to life history tradeoffs. For example, a female who reproduces for 2 seasons may adopt different reproductive options than a female that reproduces for 5 seasons, or a female who is not successful nesting one year may not nest the next year.

Theoretical work has illustrated the importance of determining precisely which factors influence adoption of reproductive options in CBP, and how frequency of reproductive options translates to larger scale population dynamics. Individual quality was a key factor in determining the frequency of reproductive options in models of CBP populations developed by Jaatinen, Lehtonen, and Kokko (2010). Eadie and Fryxell (1992) found that variations in both frequency and density dependence resulted in different ESS dynamics being responsible for maintenance of reproductive options in CBP populations. Without empirical investigations of these factors at the population and individual level, it is hard to pinpoint why female ARTS like CBP evolve and how they are maintained.

Wood ducks (*Aix sponsa*) are an excellent study species to examine factors influencing CBP over time as they are conspecific brood parasites and return to the same breeding sites in consecutive years, providing ample opportunity to examine reproductive options over multiple breeding seasons. While previous studies of CBP in wood ducks have not followed individual females across multiple years to assess consequences of the reproductive options they engage in, they have found tantalizing hints suggesting that seasonal reproductive options carry costs and benefits to future years. Wood duck females that incubate nests successfully have a higher probability of surviving to the next year (Kennamer *et al.* 2016); however, females that incubated large broods in one year were delayed nesting the next year, which could negatively influence clutch size and survival of ducklings (Rohwer & Heusmann 2007). Previous studies have only examined reproductive behaviors and their consequences for nesting females, so the long-term consequences of abstaining from nesting or only laying parasitically are completely unknown.

To fully understand CBP and other ARTs, we need to place the behaviors in their life history context by examining the frequency of reproductive options among females, determine if individuals transition between reproductive options, and explore the factors that might correlate with lifetime trajectories. In this study, we use a life history framework to examine the variation in reproductive options of wood duck females and how they are packaged over individual lifetimes. We do so at three field sites with varying densities of breeding females and available nest sites. We identify which reproductive options are present, their frequencies, and characteristics of

females adopting reproductive options within individual seasons. We then take a life history approach by examining how flexible transitions between reproductive options are over a female's lifetime. We consider three hypotheses: 1) there is no pattern to the reproductive options wood duck females employ over the course of multiple seasons, 2) females are invariant and pursue only one reproductive option over multiple years, and 3) females are flexible but follow consistent patterns in terms of the reproductive options they adopt. This study is unique in that it combines multiple robust lines of evidence (genetic parentage assignment and RFID activity at the nest) to track reproductive options adopted by known females over multiple breeding seasons to explore how alternative reproductive options are packaged to result in lifetime fitness.

## 3.2 METHODS

### 3.2.1. Study Species

Wood ducks are cavity nesters that readily nest in nest boxes, with an average clutch size of 12 eggs and a range from 1 to 58 eggs in our study populations (Odell & Eadie 2010). Male wood ducks attend females during nest site selection and mating but provide no parental care. Wood ducks are unique among North American waterfowl in that they can produce a second clutch after their first one fledges (Bellrose & Holm 1994); as such, nest boxes may reused in a season, producing multiple nesting attempts per box.

### 3.2.2. Field Methods

From 2012 to 2017, we monitored female breeding activity at 188 nest boxes at three sites in Yolo County, CA: Conaway Ranch in Woodland (n=72), Roosevelt Ranch in Zamora (n=100), and Russell Ranch in Davis (n=16). We visited nests to assess activity two to three times a week at Conaway Ranch and Russell Ranch, and weekly or bi-weekly at Roosevelt Ranch. At each visit, we counted and marked eggs with a permanent felt-tip marker, noted the identity of the incubating female if applicable, and updated the current status of the nesting attempt. This study is part of a long-term ecological research project to track wood duck reproduction, and we have records of female bands and nest activity that date back to 1998.

To obtain genotypes used for genetic assignments, we caught females on their nests between the first and third week of incubation to band them and collect a blood sample. We made additional efforts both during and after the breeding season to band and blood sample non-nesting females in nest traps and bait traps, which are designed to trap foraging ducks away from the nest. We did not include male genotypes in the study because males are not associated with nests and cannot be consistently trapped and sampled. All offspring were sampled at hatch, either by taking a blood sample or collecting eggs that did not hatch for incubation in an aviary or later dissection. Details on blood sampling, DNA extraction, and genotyping are provided in Chapter 1.

Starting in 2014, we tracked movements of breeding individuals by inserting RFID tags between the shoulder blades of all individuals during their initial handling

either as a duckling or an adult. We fitted each nest box entrance with a battery-operated RFID reader (Bridge *et al.* 2019) to record entrances and exits of RFID tagged individuals. Further details on RFID protocols are provided in Chapter 2.

### 3.2.3. Genetic Analysis

We used COLONY (Version 2.0.6.4, Jones & Wang 2010) to assign offspring to candidate mothers. COLONY assigns offspring to a candidate mother and calculates a probability associated with each assignment. COLONY can assign offspring to candidate mothers from the input female genotypes or inferred genotypes of unsampled females, so that all offspring are assigned to a female. Each duck was genotyped at 19 microsatellite loci developed for wood ducks and other closely related taxa. Our maternity analyses included females sampled in the year of interest and years prior as candidate mothers. For details on analysis parameters, see Chapter 2.

Analyses using COLONY indicated that we failed to sample the mothers of a subset of offspring at almost every site each year between 2013 and 2017 (Appendix A3.1). To attempt to identify these unsampled females, we isolated their offspring and ran another COLONY analysis, broadening the candidate parent pool to include all female offspring sampled in years prior to the one under investigation. Including these female offspring ensured that we could identify them as mothers if they returned to breed as non-nesting parasites but were never caught as adults. We also examined the nest box of origin of offspring assigned to unsampled females, as it was

possible the unsampled female was the host of a nest box if she was assigned the majority of the offspring that hatched from it. If most offspring that hatched from a nest were assigned to an unknown female, but the host's genotype was not available during our original maternity analyses (an infrequent event due to inability to sample the mother, or delay in genotype processing), we assigned those offspring to the host. Using these additional analyses, we were able to assign maternity to 121 of the 302 offspring previously assigned to unknown females and we assigned identities to 38 of the 128 previously unidentified females.

Lastly, we attempted to assign known identities to unsampled females by matching the inferred genotypes of unsampled females to those of known females. COLONY also produces inferred genotypes of unsampled females, which we attempted to match to genotypes of sampled females from the same site using CERVUS (version 3.0.7). We indicated the genotypes must match at least 18 loci with another 2 loci allowed for "fuzzy matching". This method did not successfully identify any unsampled females, suggesting that they were not simply known females that had been misidentified.

### 3.2.4. Determination of Reproductive Options, Effort, Success, and Transitions

We determined each female's reproductive option each year by examining her assigned offspring and her nesting activity. If a female was assigned only offspring in her own recorded nest, she was categorized as a nesting-only female (NO). If a female did not have a recorded nesting attempt for the year and she was assigned

offspring, we considered her a parasite-only female (PO). If a female was assigned offspring in her recorded nest as well as other nests, we considered her a nesting parasite (NP). In some cases, females were assigned only parasite offspring, but had a recorded nesting attempt that we were unable to obtain samples from; we considered these females as nesting parasites (NP).

We made note of years where females apparently were not reproductively active but had been active the year before and the year after; we considered these breeding absences (AB). We also recorded when females apparently stopped reproducing in the population altogether (EX), presumably due to death or emigration (migration between our populations appears to be infrequent; unpublished data). Since we tagged females for the first time either as ducklings or adults, and females may be engaging in different reproductive choices at different points in their reproductive trajectories, we examined the age of each female in the year prior to the first year they were assigned offspring. This allowed us to determine which tactics ducks adopted after we tagged them either as ducklings (hatch year, HY) or adults (tag year, TY).

We calculated density of breeding females for each year as the number of females that were either assigned offspring or recorded as hosting a nest divided by the area of the breeding site (in hectares) as calculated using Google Earth. We added nest boxes to some sites in later years, so we divided the number of nest boxes each year by the area of the breeding site (in hectares) to determine yearly nest box density (Appendix A3.2).

We measured reproductive effort based on the number of offspring (both hatched and unhatched) assigned to a female across all nests she contributed to, amounting to her total clutch size. We measured reproductive success by noting whether the assigned offspring successfully hatched out of its egg. Wood duck offspring leave the nest within 24-36 hours of hatching (Bellrose & Holm 1994), making tracking ducklings after they leave the nest difficult and so we could not calculate fledge rate estimates.

Once we determined tactics by season for each individual female, we combined this information across years to determine life history transitions and multi-season reproductive trajectories. We pooled sites and years to calculate frequencies of transitions between reproductive options and used a Sankey plot to examine patterns in the frequency of transitions over time.

### 3.2.5. Statistical Analysis

We conducted statistical analyses using JMP (Version 14.2.0) to examine population level patterns of frequency of reproductive options and characteristics correlated with reproductive options. To determine if reproductive options varied in frequency based on year or density of breeding females or nest boxes, we performed a mixed model with site as a random effect. We did not include breeding absences (AB) as they were extremely rare events (Appendix A3.4 and A3.5).

We determined the relationship between age and reproductive option using chi-square analyses. We ran two separate analyses: one with females tagged as

ducklings (n=100), and one with all females sampled in this study (n=275). We excluded unsampled females as we could not estimate their ages without across-year records of their activity. We calculated the minimum age of birds we captured as adults each year by subtracting the year we first captured them from the year we observed them. We know ages of birds sampled as ducklings since we know their hatch year, and we subtracted each year we observed them from their hatch year to determine their age for each year they were active.

We examined how age and reproductive option correlate with reproductive effort (as measured by eggs genetically assigned to the female) and reproductive success (as measured by the number of eggs assigned to the female that successfully hatched), using mixed models with female identity as a repeated measure. We performed a square root transform on our measures of reproductive effort and success to correct for right skew. We performed post-hoc Tukey HSD pairwise comparisons to determine differences between reproductive options.

We assessed how lifespan predicts lifetime reproductive success using simple linear regressions. We calculated lifespan as the oldest recorded age of each female. Some females were reproductively active before we started collecting genetic samples (n=23), and so we could not assign offspring to them for the entirety of their lifespan; others were first captured as ducklings in recent years, and so have not lived their full lifespan. We performed two simple linear regressions of lifetime reproductive success versus lifespan, one with all females in our study (n = 364), and one with only the females we knew had completed their lifespan (n = 181). The overall pattern did not

change and the only difference between the models was a loss of power when we restricted the females included, so we used the analysis that included all females in our study; if anything, including these females represents a conservative estimate of patterns of lifetime reproductive success, as our sample includes incomplete estimates of lifetime reproductive success.

## 3.3 RESULTS

### 3.3.1. Frequency of Reproductive Options Varies with Breeding Female and Nest Box Density

Nest box density was colinear with breeding female density, so we ran separate models to determine their relationship with the frequency of reproductive options (n = 626 reproductive option observations): one with breeding female density (AICc = 339.29) and one with nesting nest box density (AICc = 354.10). Year had no effect in either model (F = 0.75, p = 0.56 for breeding female density model, F = 1.89, p = 0.13 for nest box density model); this remained true when we excluded 2013, which was a low effort year, and 2017, which had high frequencies of unsampled mothers and consequently more females classified as only parasitizing. As breeding female density was a better predictor of frequency of reproductive options, and nest box density correlated with reproductive option frequency in essentially the same manner (Appendix A3.6), we will focus on the relationship between breeding female density and frequency of reproductive options.

The frequency of each tactic varied based on density of breeding females ($F = 17.07$, $p < .0001$). The number of females that nested without parasitizing decreased as breeding female density increased (Figure 3.1, $R^2 = 0.46$, $p = 0.006$). Although breeding female density did not have a significant relationship with and accounted for little variability in the frequency of nesting parasitism ($R^2 < 0.001$, $p = 0.95$) or parasitism without nesting ($R^2 = 0.17$, $p = 0.15$), both increased slightly with an increase in breeding female density.

### 3.3.2. Older Ducks Adopt Nesting Tactics More Frequently

When we examined yearly reproductive options (n=536 reproductive option observations) adopted by sampled females in our study populations across years, older birds adopted nesting reproductive options more frequently than the non-nesting parasite reproductive option (Figure 3.2a, $\chi^2 = 46.93$, $df = 21$, $p = 0.001$). When we examined only females we tagged as ducklings and so were of known age (n =159 reproductive option observations), there was no difference in the frequency of reproductive options adopted in different age classes (Figure 3.2b, $\chi^2 = 13.20$, $df = 9$, $p = 0.15$). We did not observe any females that parasitized without nesting beyond a minimum age of 5.

### 3.3.3. Nesting Reproductive Options Produce the Most Offspring in Single-Season Assessments

Reproductive effort ($F=22.93$, $p <0.001$) and reproductive success ($F=20.52$, $p <0.001$) varied with the reproductive option pursued by females in a single year (Figure 3.3). Females who only laid eggs parasitically had lower reproductive effort and success than nesting reproductive options ($p <0.001$), producing an average of 5 eggs (sd = ± 4) and 2 hatched offspring (sd = ± 3). Nesting reproductive options did not differ significantly in terms of reproductive effort ($p = 0.54$), with nesting parasites producing 11 eggs (sd = ± 5) on average, and females that nested without parasitizing producing 8 eggs on average (sd = ± 5). Nesting parasites had higher reproductive success than females that nested without parasitizing ($p<0.001$), with an average 8 hatched offspring (sd = ± 5) as opposed to the average 7 eggs (sd = ± 5) for females that parasitized without nesting. Reproductive effort increased with current age of the female ($F= 22.93$, $p = 0.001$, Figure 3.4). Current age did not strongly influence reproductive success ($F= 3.68$, $p = 0.06$). Female identity was the only random effect that contributed to variation in reproductive effort ($p = 0.05$), but it did not contribute to reproductive success ($p = 0.47$). Year and breeding site did not contribute to variation in reproductive effort or success.

### 3.3.4. Lifespan Predicts Lifetime Reproductive Success

Lifespan did predict variation in long-term reproductive success, with longer lifespans resulting in higher reproductive success (Figure 3.5, $R^2 = 0.28$, $p <0.001$).

### 3.3.5. Females Employ Flexible Reproductive Trajectories

Of the 364 females we tracked in our study, 60% (n=220) were only recorded breeding for a single season, and so only employed a single reproductive option. Unsampled females accounted for 40% (n=89) of the single season breeders. Of the females that reproduced for multiple seasons (n=144), 69% (n=100) employed more than one reproductive option. It was relatively rare for females to specialize in parasitic reproductive options exclusively for their whole lifetime. Out of the 44 females that employed a single option over the course of their lifetime, three exclusively parasitized without nesting and 13 only engaged in nesting parasitism. All of the non-nesting parasite specialists and 11 of the nesting parasite specialists bred only for two seasons, while the remaining two nesting parasites bred for three seasons.

Wood duck females frequently changed reproductive options across years (Figure 3.6). Females adopting nesting reproductive options (either nesting only, or nesting parasitism) most frequently either transitioned to the other nesting reproductive option, kept employing the same reproductive option, or exited the population. Nesting parasites (NP) rarely transitioned back to non-nesting parasitism (PO), and never abstained from breeding (AB) once they adopted the option. Once females transitioned out of parasitizing without nesting (PO), they rarely returned to that option. Females that abstained from breeding (AB) were most likely to transition into a nesting reproductive option, particularly nesting only (NO). Regardless of the

reproductive option they adopted, females were equally likely to exit the population each year.

The transition frequencies we observed when we pooled sites and years and are consistent with single-season ontogenetic patterns (Figure 3.7). Females reproducing for the first time after hatching (HY) disproportionally parasitized without nesting (PO). Breeding absences were rare each year. Transitions between nesting reproductive options (NO ↔ NP) were more frequent than transitions from nesting reproductive options to non-nesting reproductive options.

## 3.4 DISCUSSION

### 3.4.1. Population Frequencies of Reproductive Options

We found the number of females nesting without parasitizing decreased as breeding female density increased. We may have observed this relationship between breeding female density and the frequency of females nesting without parasitizing for a few reasons. As breeding female density increases, so does potential competition for desirable nest boxes, which may drive females that would have otherwise nested to parasitism (Semel & Sherman 2001). Additionally, an increase in breeding female density may make parasitism easier, as potential hosts are more widely available (Haramis & Thompson 1985; Rohwer & Freeman 1989) and perhaps simpler to locate by following them to their nest site (Semel *et al.* 1986; Nielsen *et al.* 2006). Our findings are consistent with previous studies of wood ducks (Haramis & Thompson 1985), goldeneye (*Bucephala islandica*, *B. clangula,* Eadie 1989), gadwall

(*Mareca strepera,* Hines & Mitchell 1984), and eider (*Somateria mollissima,* Robertson *et al.* 1992) that found that parasitism increases with increasing population density (but see McRae 1997).

### 3.4.2. Single-Season Correlates of Reproductive Options

Based on annual estimates of individual female reproductive success, we found older wood duck females are more likely to adopt nesting reproductive options. Older age was also correlated with higher reproductive effort (the production of more eggs), regardless of the reproductive option adopted, suggesting that older females may have the ability to access to more resources, resulting in increased egg production. Although older age resulted in higher reproductive effort across reproductive options, as assessed by total eggs laid in a year, there were differences in average reproductive effort between reproductive options. Parasitizing without nesting was lower effort compared to nesting reproductive options (nesting and parasitizing, or nesting without parasitizing), both of which have higher average single-season reproductive effort. Combined with age related patterns, it appears that younger wood duck females tend to be parasites that exclusively lay in other female's nests, producing a relatively small clutch size. This is consistent with other studies of CBP waterfowl (Sorenson 1993a; Semel & Sherman 2001; Lyon 2003).

Previous studies have suggested that individual quality might be a major factor determining CBP reproductive options (Sorenson 1993b; Lyon & Eadie 2008; Jaatinen *et al.* 2010), but the fact that female identity did not influence differences in

single-season estimates of reproductive success suggests that the role of individual quality factors in strongly over the course of a lifetime trajectory, rather than in a season-by-season basis. We found that as females age, they are more likely to adopt nesting reproductive options that result in higher average egg production and hatch success, although we were unable to examine the role of individual quality in this pattern, which may have important implications for reproductive trajectories. Previous theoretical and empirical literature suggests that females only adopt nesting reproductive options as they obtain resources that allow them to allocate more effort into egg production (Lyon & Eadie 2008). Therefore, high quality females may be able to transition into nesting reproductive options earlier, maximizing lifetime reproductive success. Teasing apart the role of age versus individual quality as they relate to reproductive success will be a rich area of focus for future studies of CBP.

The nesting reproductive options did not differ significantly in terms of single-season reproductive effort, but nesting parasites had higher single-season reproductive success than females that nested without parasitizing. This pattern of reproductive success has been observed several times in studies of CBP (Lyon 1993; McRae 1997b; Ahlund & Andersson 2001; Lyon & Eadie 2018), and makes sense as parasitic offspring provide a reproductive bonus for nesting parasites. If nesting parasites are able to achieve the same hatch success as a nesting only female in their own nest, even if hatch success of their parasitic offspring was lower, as often observed (Lyon 1998; Šťovíček *et al.* 2013), any additional parasitic young that do hatch would improve the reproductive success of nesting parasites over nesting

females who do not parasitize. Although generally infrequent in our study populations, nest abandonment, depredation, and failure result in zero net reproductive success for females who nest without parasitizing (Appendix A3.3). Nesting parasitism allowed females that adopted it to achieve some reproductive success when their nests failed; 15 nesting parasites achieved reproductive success entirely from their parasitic offspring during at least one season we observed, due to nest abandonment or depredation of their own nest, again giving nesting parasites a reproductive advantage through their parasitic offspring.

The patterns we uncovered with regards to single season reproductive effort and success were sensitive to the individuals we included in the analysis and to the parameters of the analysis we used. For instance, we did not find significant age-related differences in frequency of reproductive options when we restricted our analysis to only include females of known age; this is likely because we have only followed cohorts of ducklings for four years, which may not be enough time to detect age related differences in reproductive options. Additionally, our sample size may have lacked the statistical power necessary to detect those differences. We ran our analyses of reproductive effort and success across reproductive options with different subsets of females and different transformations, and while the variables of influence remained consistent across analyses, the pairwise differences between reproductive options changed in significance, particularly between the nesting options. Additional years of data and an increased sample size of known-age ducklings might solidify these patterns statistically. Alternatively, the differences between reproductive

options may not be as distinct in our populations as in other studies, since age-related increases in overall reproductive effort may blur the distinction between reproductive options when all age classes are combined to make single-season estimates of reproductive effort and success. Comparing reproductive effort and success within age groups may produce more defined differences between reproductive options, which would shed light onto the potential age-specific costs and benefits of adopting a reproductive option.

### 3.4.3. The Context of Lifetime Transitions and Trajectories

Considering reproductive options in the context of multiple seasons provided important insights into how reproductive effort and success accrue over a lifetime. Once females transitioned to a nesting reproductive option (NO or NP), they rarely transitioned back to non-nesting options. Combined with the ontogenetic patterns observed from our single season analyses, it appears that females age into nesting reproductive options and stay there, perhaps switching between laying additional eggs parasitically and not parasitizing, until they exit the population. This suggests that a key factor to higher lifetime reproductive success simply lies in survival to older ages, which correlates with in an increase in reproductive effort and, for nesting reproductive options, reproductive success.

Females rarely specialized in any one reproductive option their entire lifetime. A lifetime specialization in parasitizing without nesting has been proposed as a theoretical possibility (Jaatinen *et al.* 2010), but no empirical studies have found

support that females do this (Lyon 1993; Reichart *et al.* 2010; Lyon & Eadie 2018). We found scant evidence for lifetime specialization in general: females that only adopted one reproductive option their whole life were in the minority of our female population, and it was rarer still for females to specialize in parasitic reproductive options. No lifetime parasite specialists were recorded in our populations for more than 3 years in a row. There is the possibility we did not record their full lifespan, or possibly missed nests and misidentified females as parasitizing without nesting when they actually did have a nest. Still, the high frequency of females that adopted multiple reproductive options over their lifetimes indicates that flexibility of reproductive options is a key component in optimizing reproductive output. While reproductive effort increases with age, it is likely that individual trajectories are important in explaining variation in performance of reproductive options as a whole. Previous studies have suggested that individual quality might be a major determining factor of CBP reproductive options (Sorenson 1993b; Lyon & Eadie 2008; Jaatinen *et al.* 2010). Our next steps will be to address variation in individual trajectories formally by using Markov-Chain Monte Carlo methods to see which trajectories are most frequently observed, and then examining the relationship of these trajectories to variation in lifetime reproductive success.

All reproductive options had equal annual rates of exiting the population, which suggests that no one reproductive option has a survival advantage. However, there may be an interaction between age, reproduction option, and survival that determines when females are most likely to exit. The overall trajectories females

choose may determine the age that they exit the population, but that analysis is beyond the scope of this paper.

We did not have full lifetime reproductive effort or success for many of the females in our study populations, as many of our birds were caught as ducklings and have not completed their lifetimes, or they were caught before we started sampling genetics and so we could not assign offspring for those years. Consequently, our estimates of reproductive effort and success are conservative. This may mean we are underestimating the role of the parasite-only reproductive option, as that tended to be adopted more frequently earlier in an individual's breeding trajectory. However, another part of our population was only sampled at the start of their breeding trajectory (the ducklings hatched from our populations), and may have compensated for at least single-season estimates of reproductive effort and success.

Ultimately, putting reproductive options in the context of an individual's lifetime is essential. Even if a single-season estimate is good approximation of how reproductive options contribute to lifetime reproductive success, lifetime fitness is a sum of reproductive success over the course of a lifetime. Understanding how flexible reproductive trajectories are and the reproductive consequences of transitions between reproductive options is an important basis from which to launch more detailed investigations of CBP behavior. Now that we have an increased understanding of the frequency and flexibility of these reproductive options and have some measure of their success both within seasons and in terms of lifetime trajectories, we can examine more thoroughly the costs and benefits of adopting

reproductive options, including how tradeoffs among them may influence lifetime trajectories and reproductive success.

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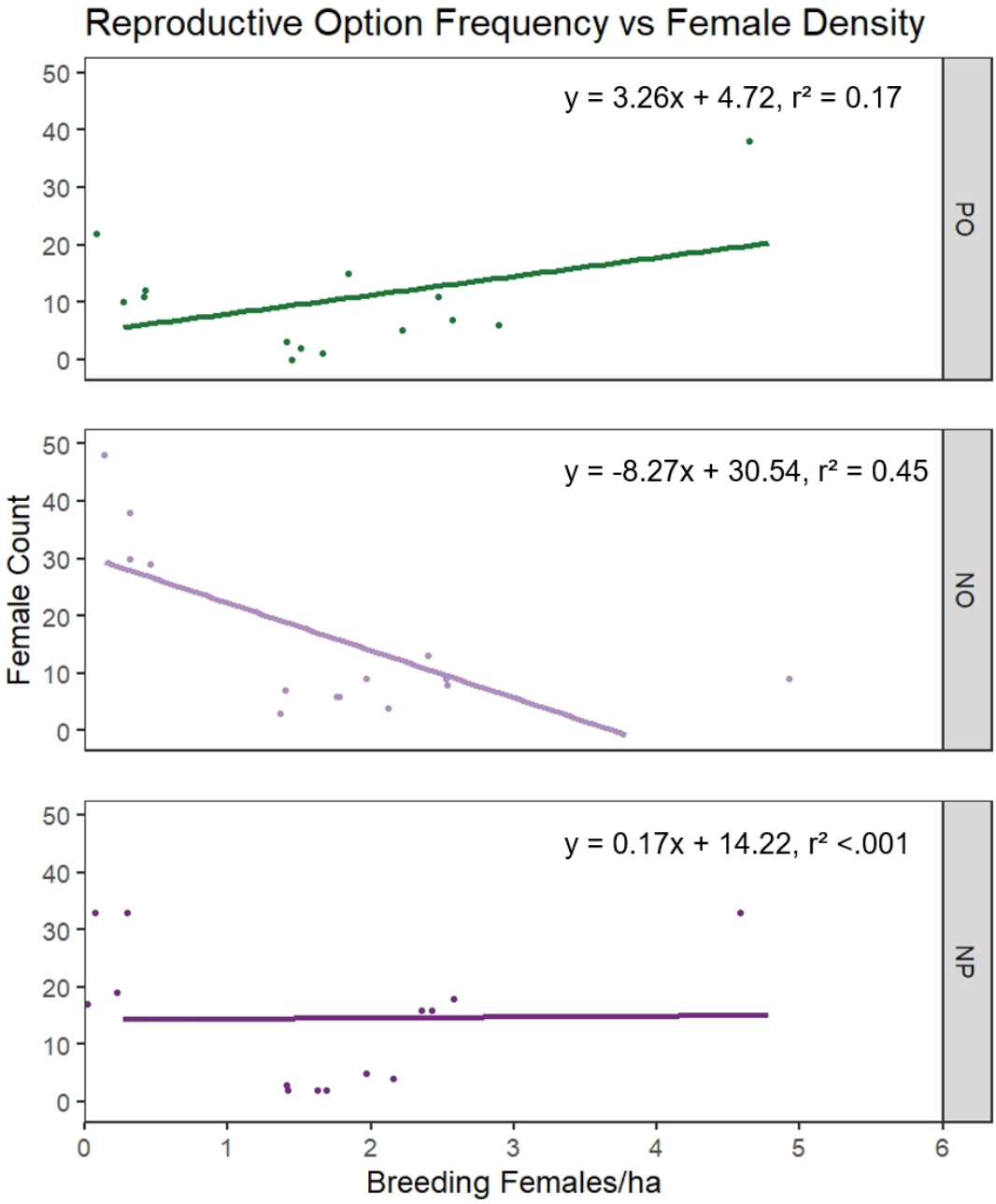



**Figure 3.1.** The frequency of reproductive options in relation to the density of breeding females (as detected by genetics or nest records) per hectare at the three field sites included in this study. Sites and years are pooled.

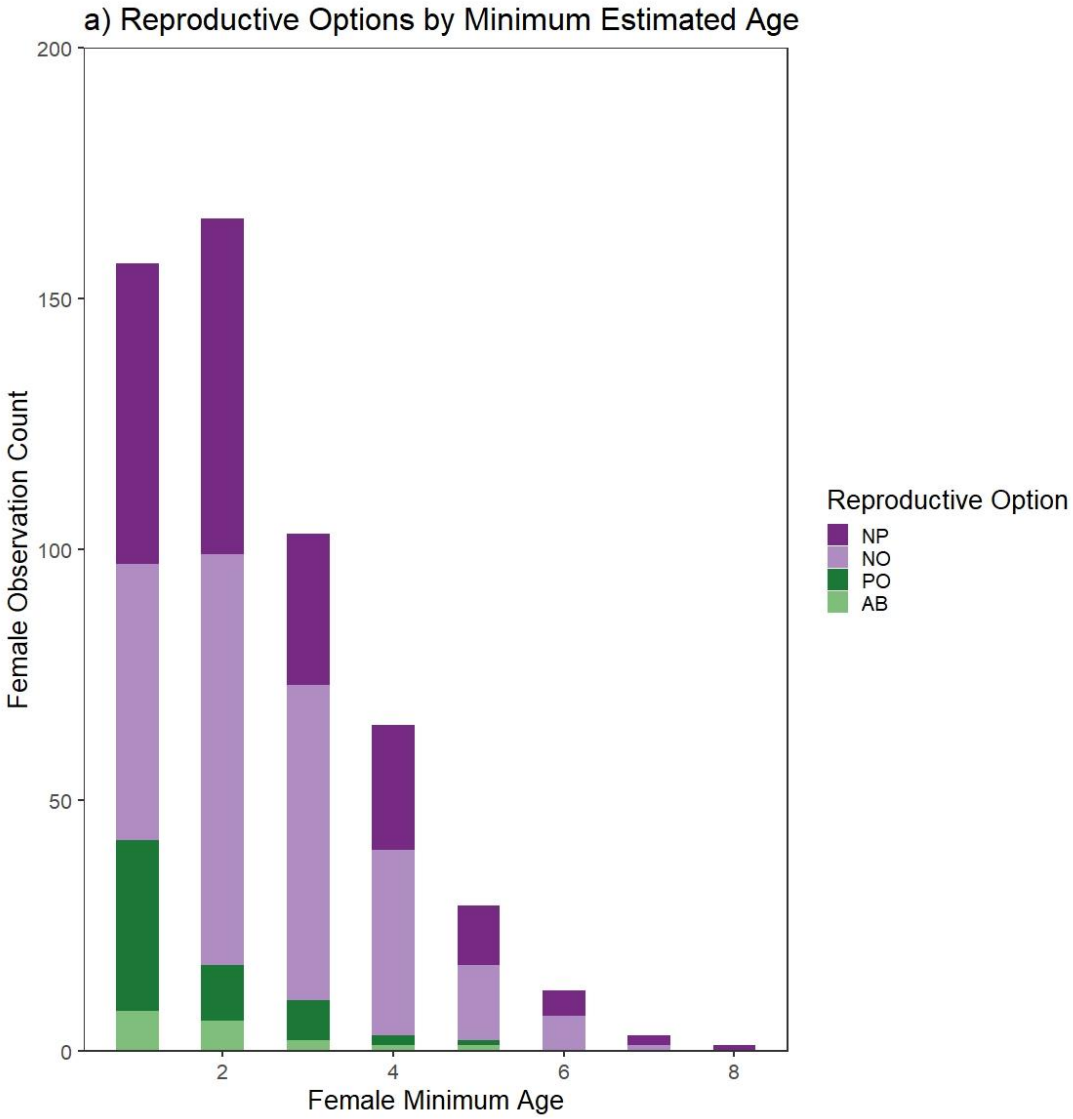



**Figure 3.2.A.** Reproductive option distribution for all sampled females in our populations. The counts are measured in observations of female reproductive options in the age category listed, so females active more than one season are represented in multiple age classes. We calculated minimum age based on estimated age at capture plus how many years had elapsed since we first caught the female. The reproductive option classes included are nesting parasitism (NP), nesting without parasitizing (NO), parasitizing without nesting (PO), and abstaining from breeding (AB).

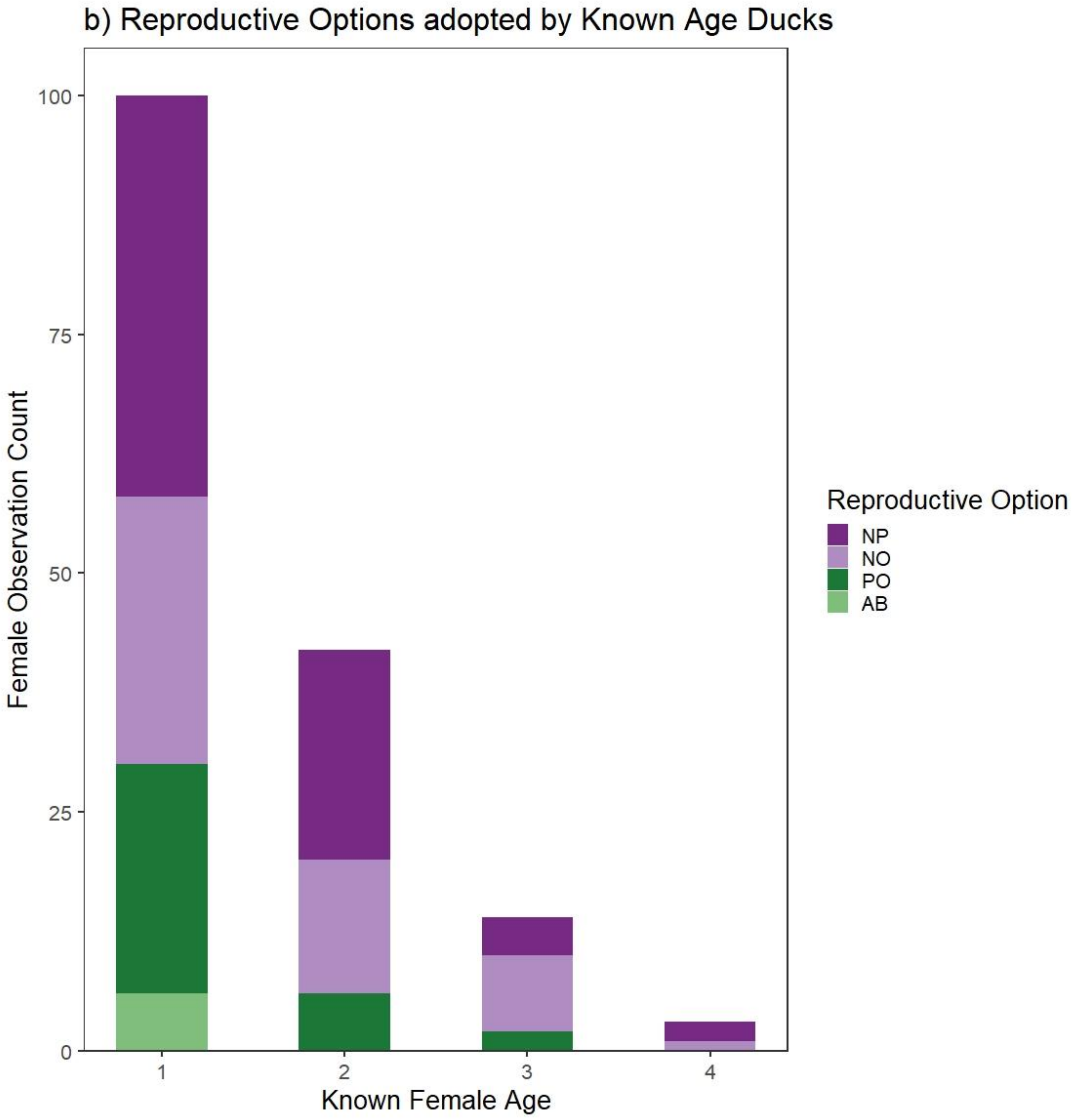



**Figure 3.2.B.** Reproductive option distribution for females caught as ducklings, and so we are certain of their age. The counts are measured in observations of female reproductive options in the age category listed, so females active more than one season are represented in multiple age classes. The reproductive option classes included are nesting parasitism (NP), nesting without parasitizing (NO), parasitizing without nesting (PO), and abstaining from breeding (AB).

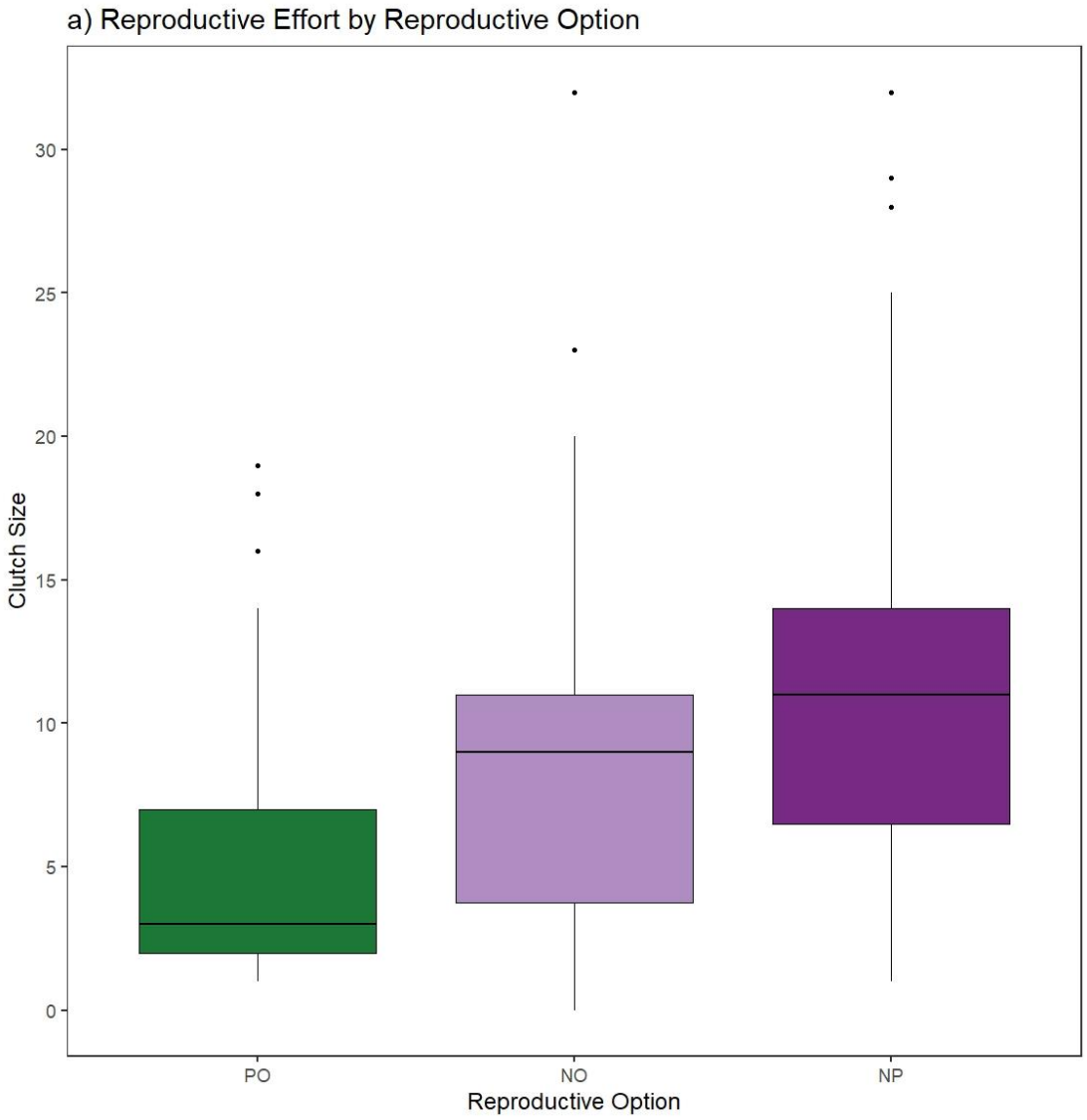



**Figure 3.3.A.** The relationship between reproductive effort (as determined by genetically assigned offspring) and reproductive option of the female. Reproductive effort was calculated for females that nested and parasitized (NP), nested without parasitizing (NO), and parasitized without nesting (PO). All sites and years were pooled. The interquartile range of each boxplot represents the middle 50% of count observations for each reproductive option, with each whisker representing an additional 25% of count observations above or below the interquartile range. The median value for each reproductive option is marked by the black horizontal line.

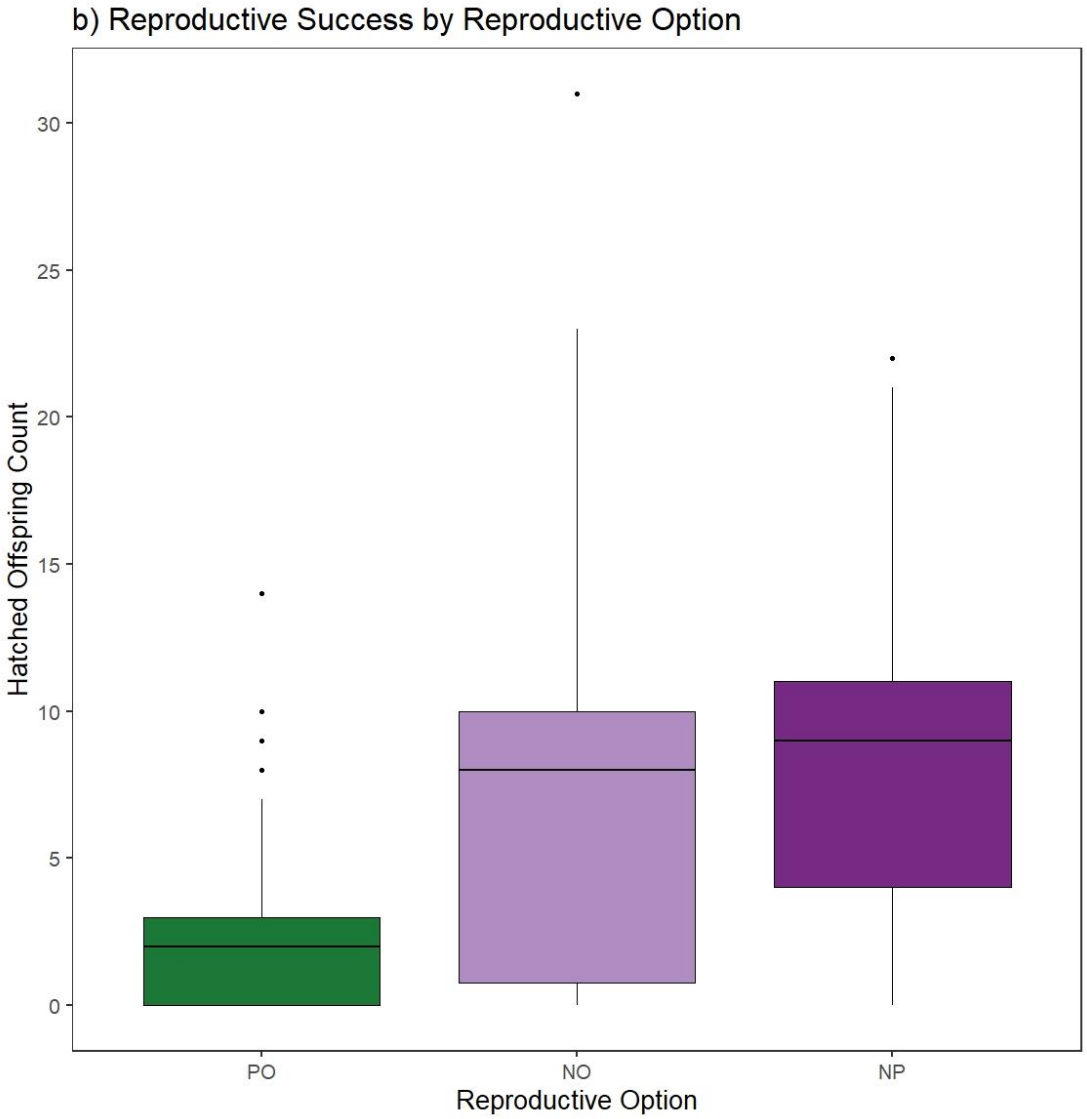



**Figure 3.3.B.** The relationship between reproductive success (as determined by genetically assigned offspring that successfully hatched) and reproductive option of the female. Reproductive success was calculated for females that nested and parasitized (NP), nested without parasitizing (NO), and parasitized without nesting (PO). All sites and years were pooled. The interquartile range of each boxplot represents the middle 50% of count observations for each reproductive option, with each whisker representing an additional 25% of count observations above or below the interquartile range. The median value for each reproductive option is marked by the black horizontal line.

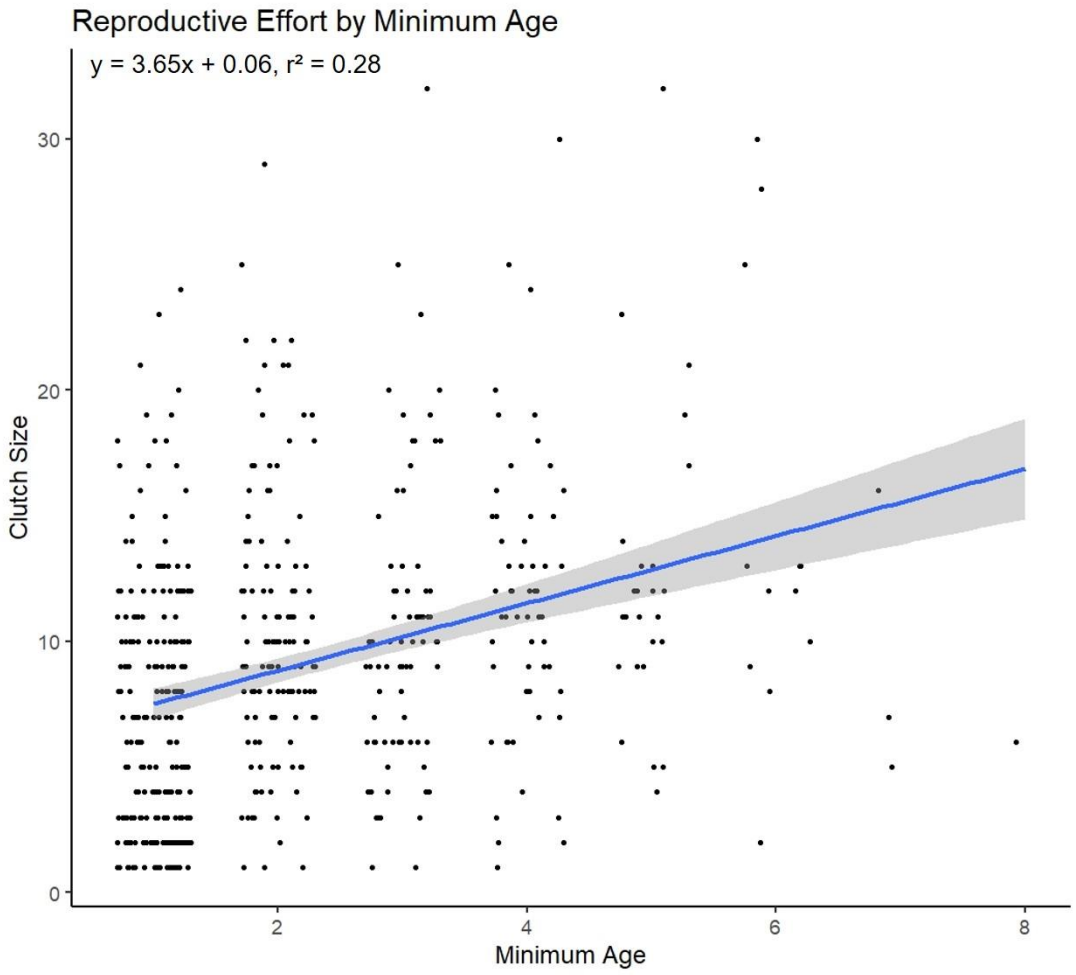



**Figure 3.4.** The relationship between reproductive effort (as measured by clutch size assigned or attributed to a female) and the minimum recorded age of the female (n = 526 observations). Sites, years, and reproductive option categories are pooled. The shaded gray region indicates the 95% confidence interval.

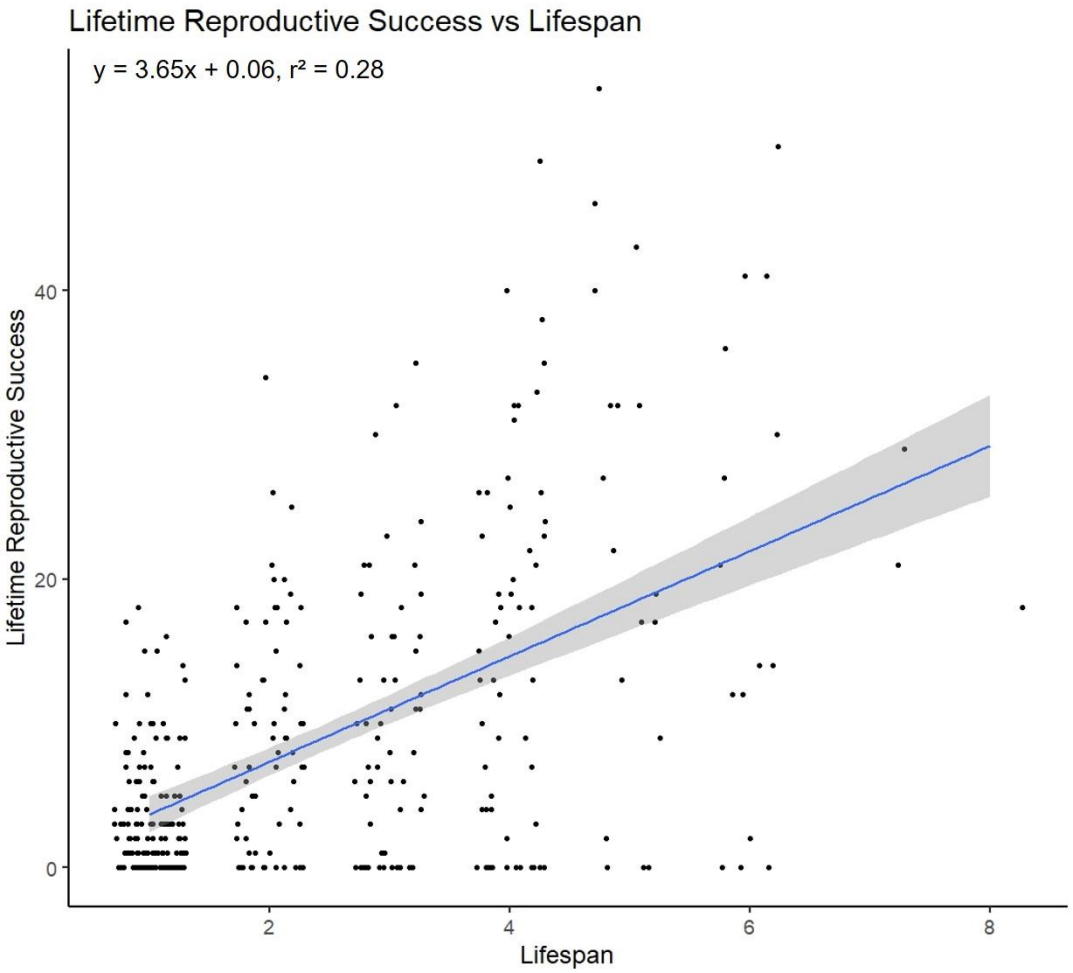



**Figure 3.5.** The relationship between lifetime reproductive success (measured in hatched offspring) and lifespan (measured in years).

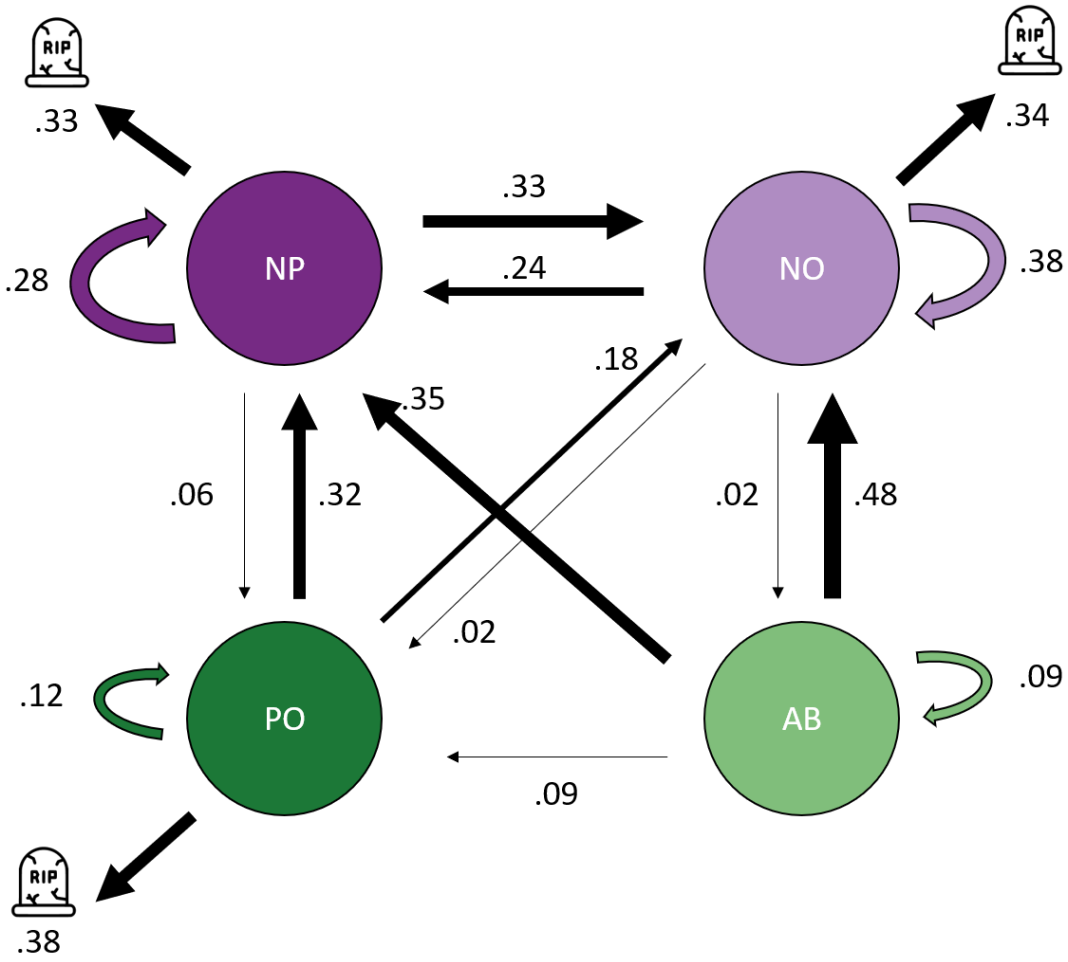
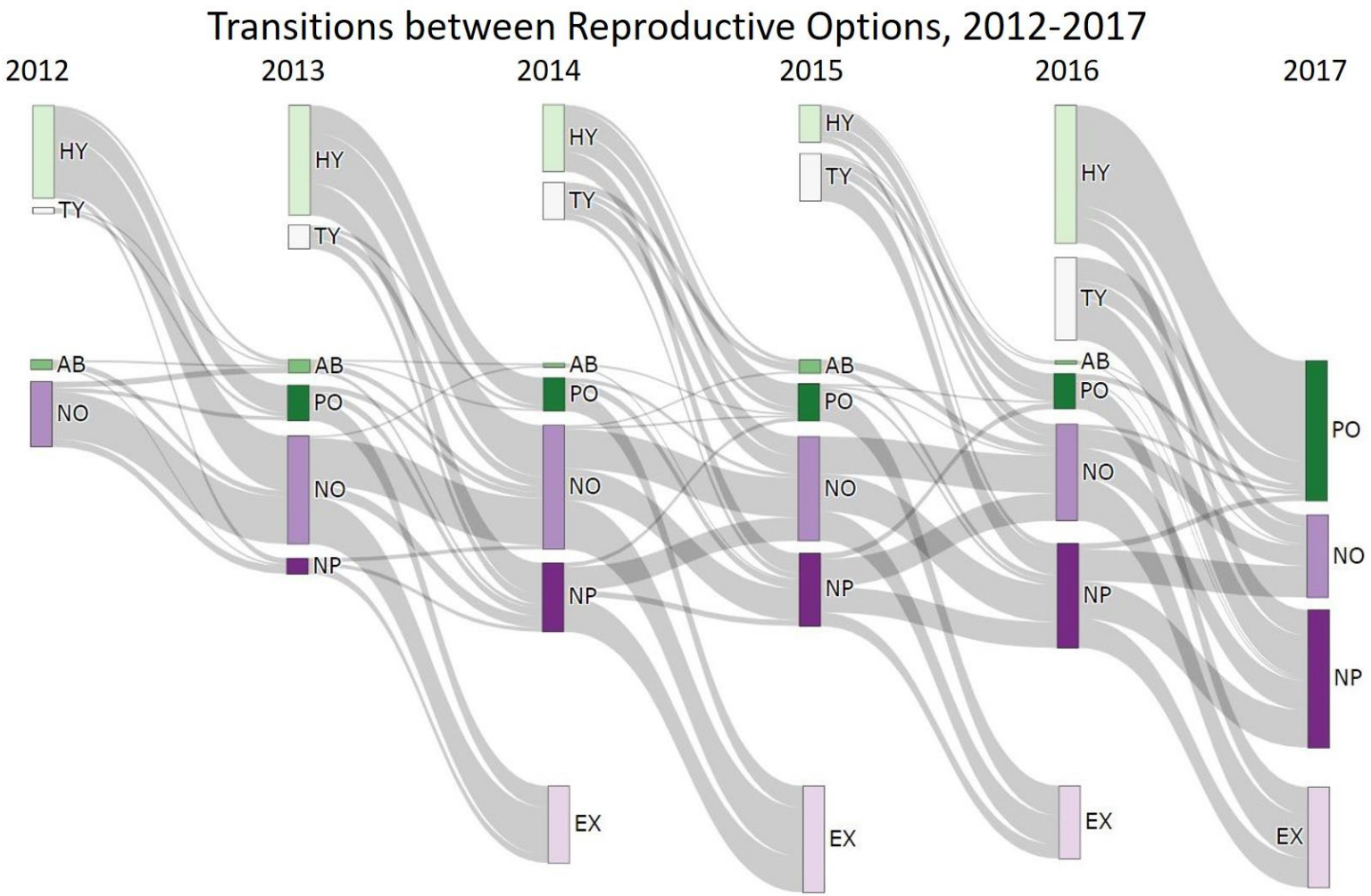



**Figure 3.6.** Transitions (n = 773) between reproductive options, 2012-2017. All sites and years were pooled, and all females including unsampled females were included in the transition totals (n = 364). The reproductive options are nesting and parasitizing (NP), nesting without parasitizing (NO), parasitizing without nesting (PO), abstaining from breeding (AB), and exiting the population (represented by gravestones).

**Figure 3.7.** Sankey plot displaying transitions between reproductive options employed by females (n=364) over time. The initial entrances into the population are separated into females we marked as ducklings (HY, hatch year females) and females we initially tagged as adults (TY, tag year females). The reproductive options are nesting and parasitizing (NP), nesting without parasitizing (NO), parasitizing without nesting (PO), and abstaining from breeding. Exits from the population are labelled EX.


Transitions between Reproductive Options, 2012-2017
2012
2013
2014
2015
2016
2017
HY
TY
AB
NO
PO
NP
EX

# SYNTHESIS

## METHODS OF DETECTING CONSPECIFIC BROOD PARASITISM

My dissertation established that genetic methods of maternity assignment provide accurate estimates of conspecific brood parasitism (CBP), despite incomplete parent sampling and related individuals in the candidate parent pool. Genetic methods were most likely to misrepresent CBP by assigning parasitic offspring of non-nesting females to females present in the candidate parent pool or not assigning them to any candidate female, making it so non-nesting parasitism goes under-detected using these methods. Non-genetic methods of parentage assignment also tended to provide underestimates of parasitism, particularly non-nesting parasitism; as such, it seems that it is most likely that parasitism has been undetected or under-detected up to this point. Given the error rates I observed in both non-genetic and genetic methods of assessing CBP, it seems unlikely CBP has been greatly underestimated, which is reassuring as even small underestimates might have larger consequences depending on the question being asked. The frequency of parasitism could have important consequences for management and conservation in CBP species (Lyon & Eadie 2008), as previous theoretical work has shown that not only the frequency but the type of parasitism can have consequences for population stability (de Valpine & Eadie 2008). To date, few studies have empirically examined the influence of CBP on population dynamics, but accurate assessments of parasitism will be essential to truly understand how the frequency of each reproductive option influences population dynamics. At the level of individual females, a reduction in detecting parasitic

offspring could result in an underestimate of reproductive effort and/or success for parasite females, which would misrepresent the fitness consequences of adopting reproductive options, leading to false conclusions about the role of parasitic behavior in CBP (Lyon & Eadie 2018).

Although genetic methods of assigning maternity are largely robust on their own, non-genetic methods provide essential context as to how CBP operates. Non-genetic methods in combination with genetic methods provided clues to patterns in parasitic laying, which can help focus hypothesis testing in future research efforts. Additionally, multiple lines of evidence lead to stronger support of patterns, and so it is still important to analyze non-genetic evidence of maternity to ensure patterns of parasitism are robust. With the advent of remote sensing technologies like radio frequency identification (RFID) and geo-logging, vast quantities of rich behavioral data are easier to collect than ever before and can integrate with genetic sources of maternity to provide invaluable insights into CBP and other ARTs.

## LONG TERM PATTERNS OF CONSPECIFIC BROOD PARASITISM

I found that female wood ducks flexibly transition between CBP reproductive options between seasons and throughout their lifetimes, consistent with previous research on other male and female ARTs (Caro & Bateson 1986; Eadie 1989; Hill *et al.* 2015). Females that adopted only one reproductive option throughout their lifetimes were rare, and virtually no females were lifetime parasites. While nesting parasitism produced the most offspring seasonally and contributed the most offspring

to overall lifetime reproductive success, there was enough individual variation in reproductive success that parasitism or nesting alone could result in high reproductive effort, which often translated to high reproductive success. As suggested by previous theory and research (Sorenson 1993; Lyon & Eadie 2008; Jaatinen *et al.* 2010), individual quality may hold the key to explaining much of the observed variation in effort and success in wood ducks, so taking a focused look at how and when individual females vary in their reproductive options is critical to understand the reason why CBP reproductive options are maintained in populations.

Lifetime reproductive success was at least partly explained by lifespan in wood ducks, with longer-lived birds producing more hatched offspring over their lifetimes than shorter-lived birds. This pattern could be due in part to the ontogenetic signal of aging into nesting reproductive options. Nesting females are older, and older birds produce more eggs, which translates to higher seasonal reproductive success. Achieving higher lifetime reproductive success thus seems to be a matter of surviving to older ages, when adopting nesting reproductive options is more common.

The pattern of increasing reproductive effort with age may also be an indication of why younger birds invest less in starting their own nests and most frequently only engage in parasitism – they are either incapable of doing so until an older age, or reserve that ability until they are older and able to nest with better success. Although I found that females that only engage in parasitism do poorly in terms of single-season averages, which is consistent with other studies of CBP, it seems that engaging in parasitism without nesting is an important stage in many

females' reproductive trajectories, particularly when they initially start reproducing. Therefore, parasites only might not only be doing best of a bad job but reserving their reproductive resources for a time when they may achieve higher reproductive success. Additionally, it is important to keep in mind that despite apparent poor performance compared to nesting reproductive options, pure parasitism is still an increase in fecundity for a female whose alternative option is to abstain from reproduction entirely (Lyon & Eadie 2018).

## FUTURE DIRECTIONS

Long-term studies add vital context to our knowledge of the evolution and maintenance of CBP and other ARTs. While organisms make reproductive decisions in the context of one season, the cumulative result of those reproductive choices is lifetime reproductive success, the ultimate fitness measurement of how well a phenotype performs and which traits are carried into future generations. As such, single season estimates are useful snapshots, but only tell a part of the story of an organism's reproductive trajectory and success. Transitions between reproductive options can highlight the most important factors that could explain variation in reproductive behaviors between individuals, and why diversity in reproductive behaviors is continually maintained even when some options are apparently not as productive as others. Future studies should take a long-term life history based approach to understanding why females display the range of behaviors they do flexibly over the course of their lifetimes, starting with examining individual female

patterns of multi-season trajectories and correlates with transitions between reproductive options.

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# APPENDICES

## APPENDIX A1: Supplemental Materials for Chapter 1

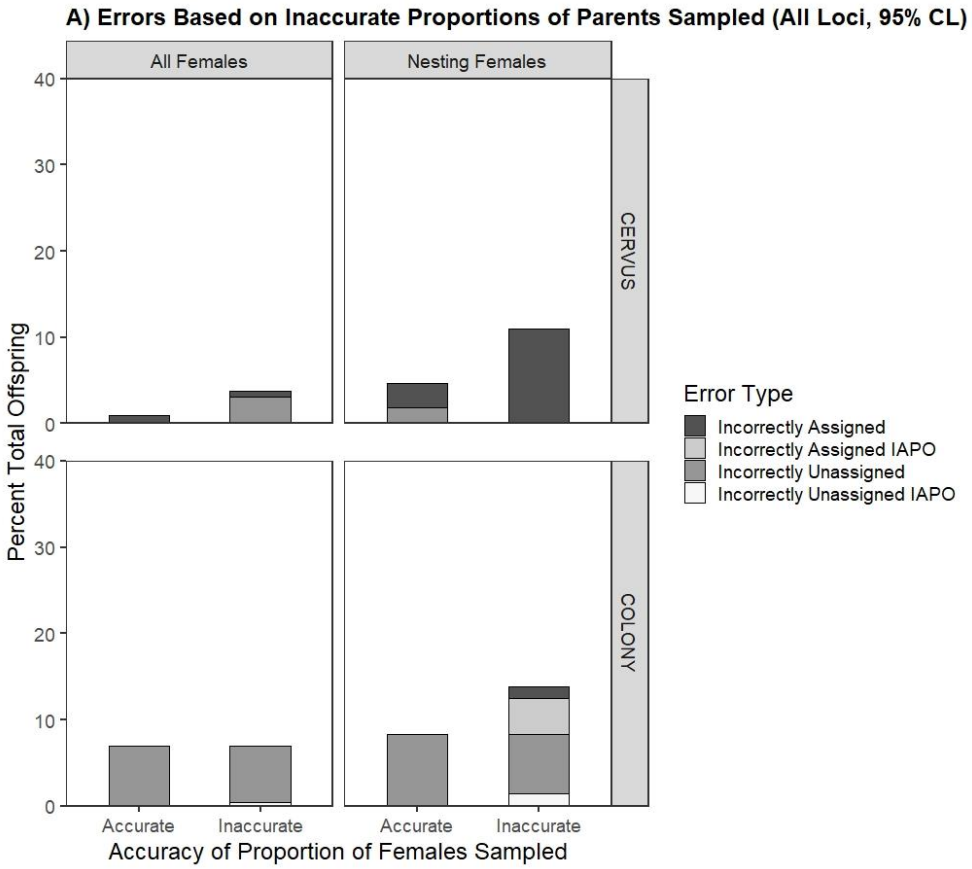
**Appendix A1.1.** Autosomal markers used in simulating female and duckling wood ducks for the 1) Conaway Ranch population and 2) Roosevelt Ranch population. Polymorphic information content (PIC) is a measure of informativeness of each locus calculated from allele frequency analysis. The Half Loci column indicates whether a locus was included in our analyses that only used 9 loci. DNA was extracted from samples using DNeasy Blood and Tissue Kit spin columns (Qiagen, USA) according to manufacturer's protocol, or using a plate-extraction method (https://github.com/WhiteheadLab/Bench_Protocols/blob/master/Ampure_DNA_extraction.md; substituting 2mm punch from blood strip for the fin clip).

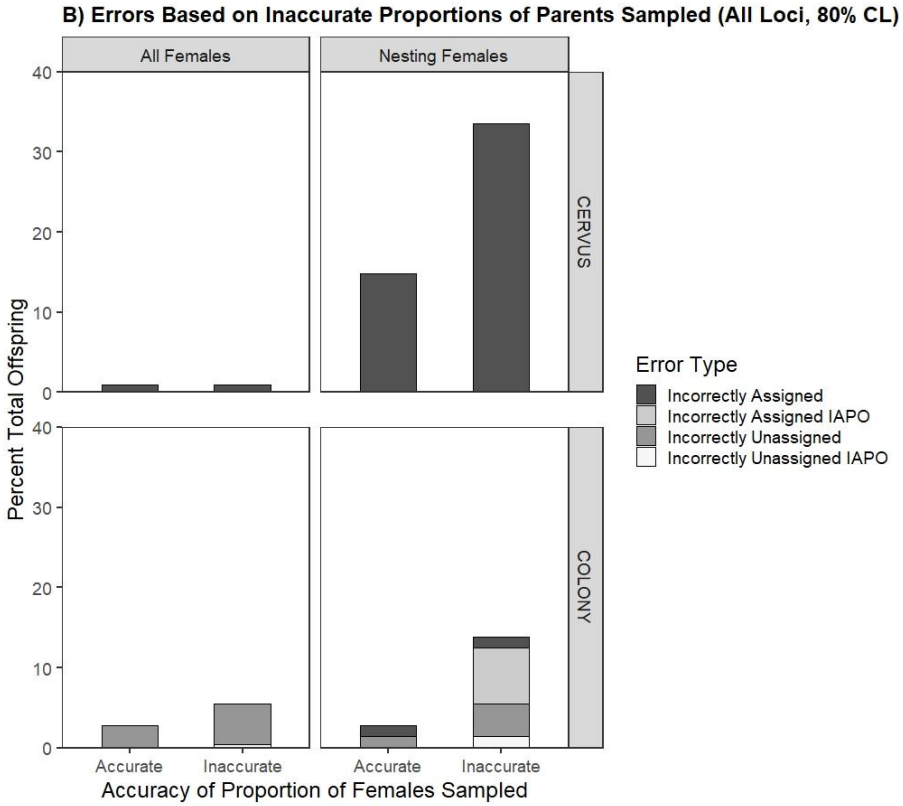
**Appendix A1.1.1.** Conaway Ranch microsatellite markers.

| Marker | Source | Allele Size Range | # Alleles | $H_o$ | PIC | Half Loci |
|---|---|---|---|---|---|---|
| **APH01** | Maak *et al*. 2000; Maak *et al*. 2003 | 204-206 | 2 | 0.52 | 0.42 | N |
| **APH02** | | 98-112 | 8 | 0.79 | 0.71 | N |
| **APH08** | | 99-115 | 4 | 0.57 | 0.53 | N |
| **APH09** | | 96-102 | 4 | 0.29 | 0.30 | N |
| **APH13** | | 170-174 | 5 | 0.77 | 0.71 | Y |
| **APH19** | | 162-168 | 4 | 0.58 | 0.52 | Y |
| **APH20** | | 143-148 | 4 | 0.63 | 0.65 | Y |
| **APH23** | | 179-192 | 5 | 0.69 | 0.57 | Y |
| **APH25** | | 177-197 | 7 | 0.66 | 0.63 | Y |
| **APL02** | Denk et al. 2004 | 108-110 | 2 | 0.31 | 0.27 | N |
| **APL23** | | 103-118 | 5 | 0.61 | 0.51 | Y |
| **BCA05** | Buchholz et al. 1998 | 171-174 | 3 | 0.21 | 0.20 | Y |
| **CM28** | Stai and Hughes 2003 | 187-256 | 26 | 0.96 | 0.94 | Y |
| **CM35** | | 215-269 | 11 | 0.75 | 0.78 | N |
| **SFI04** | Fields and Scribner 1997 | 142-144 | 2 | 0.47 | 0.34 | N |
| **SMO04** | Paulus and Tiedemann 2003 | 160-434 | 36 | 0.96 | 0.95 | Y |
| **SMO07** | | 182-188 | 3 | 0.50 | 0.40 | N |
| **SMO10** | | 6-105 | 7 | 0.59 | 0.49 | N |

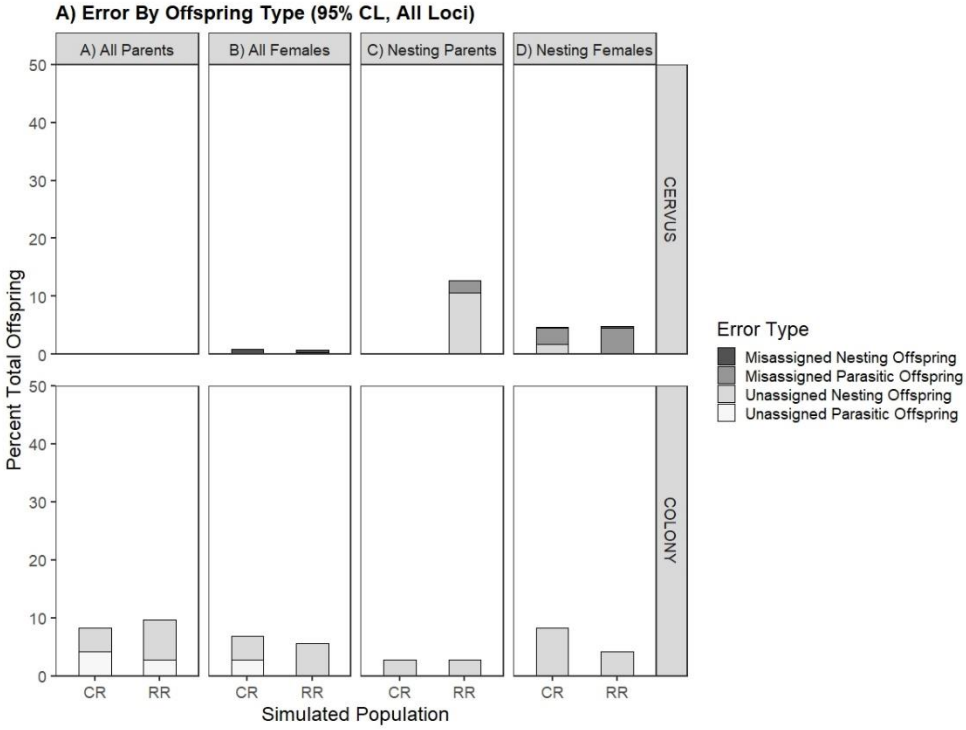
**Appendix A1.1.2.** Roosevelt Ranch microsatellite markers.

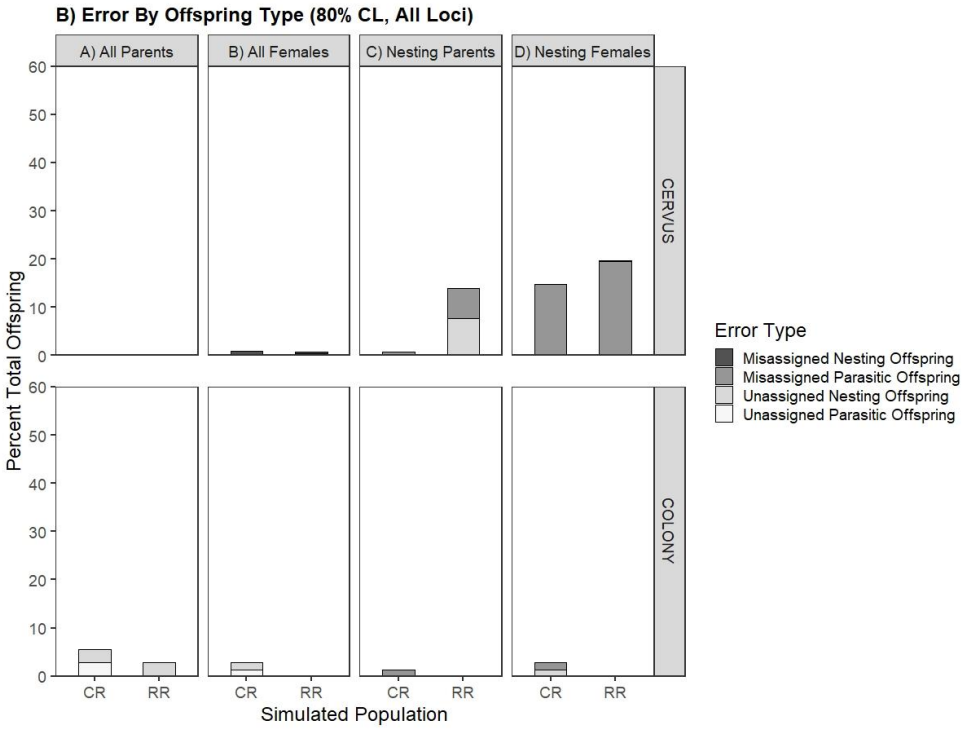
| Marker | Source | Allele Size Range | # Alleles | $H_o$ | PIC | Half Loci |
|---|---|---|---|---|---|---|
| **APH01** | Maak *et al*. 2000; Maak *et al*. 2003 | 204-206 | 3 | 0.55 | 0.42 | N |
| **APH02** | | 98-116 | 8 | 0.79 | 0.76 | N |
| **APH08** | | 99-115 | 4 | 0.60 | 0.52 | N |
| **APH09** | | 94-100 | 4 | 0.35 | 0.27 | N |
| **APH13** | | 170-174 | 5 | 0.76 | 0.69 | Y |
| **APH19** | | 162-168 | 4 | 0.62 | 0.52 | Y |
| **APH20** | | 143-148 | 5 | 0.69 | 0.57 | Y |
| **APH23** | | 179-192 | 6 | 0.63 | 0.58 | Y |
| **APH25** | | 185-215 | 8 | 0.71 | 0.62 | Y |
| **APL02** | Denk et al. 2004 | 108-110 | 2 | 0.32 | 0.29 | N |
| **APL23** | | 103-118 | 5 | 0.58 | 0.52 | Y |
| **BCA05** | Buchholz et al. 1998 | 171-200 | 5 | 0.21 | 0.21 | Y |
| **CM28** | Stai and Hughes 2003 | 163-259 | 26 | 0.93 | 0.94 | Y |
| **CM35** | | 215-269 | 11 | 0.82 | 0.71 | N |
| **SFI04** | Fields and Scribner 1997 | 142-144 | 2 | 0.48 | 0.36 | N |
| **SMO04** | Paulus and Tiedemann 2003 | 160-434 | 38 | 0.96 | 0.95 | Y |
| **SMO07** | | 182-188 | 3 | 0.50 | 0.42 | N |
| **SMO10** | | 93-115 | 7 | 0.55 | 0.51 | N |

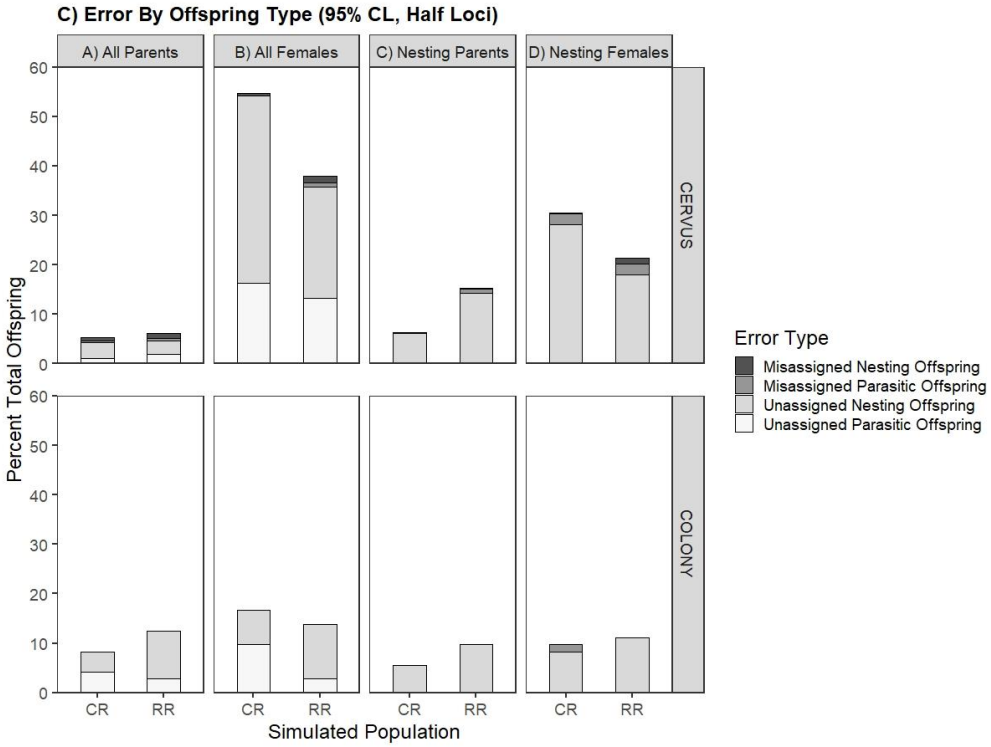

A) Errors Based on Inaccurate Proportions of Parents Sampled (All Loci, 95% CL)
All Females
Nesting Females
CERVUS
COLONY
Percent Total Offspring
0
10
20
30
40
Accurate
Inaccurate
Accuracy of Proportion of Females Sampled
Error Type
Incorrectly Assigned
Incorrectly Assigned IAPO
Incorrectly Unassigned
Incorrectly Unassigned IAPO

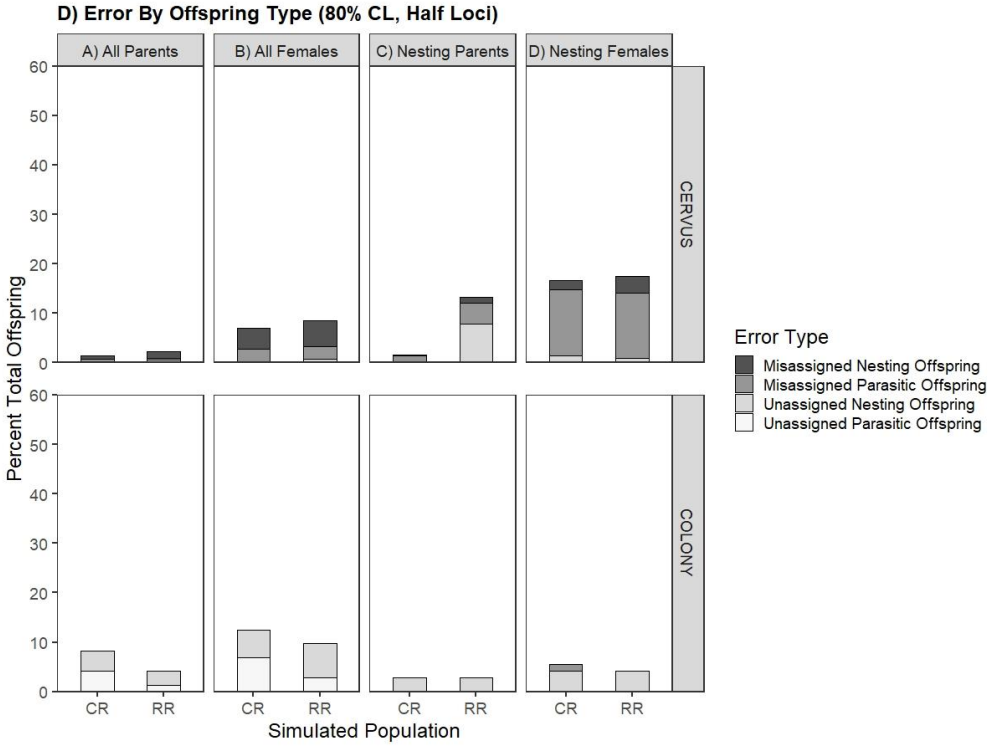



**Appendix A1.2.** We ran analyses in which we included all females in the candidate parent pool but told the programs we only sampled 67% of the population (an underestimate of the actual candidate parent pool), and analyses where we only included nesting females but told the programs we sampled 100% of the females (an overestimate of the candidate parent pool). We examined the results using either a A) 95% confidence level or B) 80% confidence level. Overestimating the number of females sampled led to an overinflated level of confidence for both programs, and consequently more incorrect assignments. An underestimate of females assumed sampled led to a decrease in confidence, which increased the number of offspring left erroneously unassigned. These analyses were based on 18 loci using the Conaway Ranch simulated population. Darker colors indicate that the identity of the assigned female did not change with the change in proportion of females sampled but the program assigned confidence level did. The light colors indicate that the identity of the assigned female changed, or the type of assignment changed with a change in proportions assumed sampled (IAPO stands for inaccurate proportions only).


A) Error By Offspring Type (95% CL, All Loci)
A) All Parents
B) All Females
C) Nesting Parents
D) Nesting Females
CERVUS
COLONY
Percent Total Offspring
0
10
20
30
40
50
CR
RR
Simulated Population
Error Type
Misassigned Nesting Offspring
Misassigned Parasitic Offspring
Unassigned Nesting Offspring
Unassigned Parasitic Offspring


B) Error By Offspring Type (80% CL, All Loci)
A) All Parents
B) All Females
C) Nesting Parents
D) Nesting Females
CERVUS
COLONY
Percent Total Offspring
0
10
20
30
40
50
60
CR
RR
Simulated Population
Error Type
Misassigned Nesting Offspring
Misassigned Parasitic Offspring
Unassigned Nesting Offspring
Unassigned Parasitic Offspring


C) Error By Offspring Type (95% CL, Half Loci)
A) All Parents
B) All Females
C) Nesting Parents
D) Nesting Females
CERVUS
COLONY
Percent Total Offspring
0
10
20
30
40
50
60
CR
RR
Simulated Population
Error Type
Misassigned Nesting Offspring
Misassigned Parasitic Offspring
Unassigned Nesting Offspring
Unassigned Parasitic Offspring


D) Error By Offspring Type (80% CL, Half Loci)
A) All Parents
B) All Females
C) Nesting Parents
D) Nesting Females
CERVUS
COLONY
Percent Total Offspring
0
10
20
30
40
50
60
CR
RR
Simulated Population
Error Type
Misassigned Nesting Offspring
Misassigned Parasitic Offspring
Unassigned Nesting Offspring
Unassigned Parasitic Offspring

**Appendix A1.3.** Assignment errors sorted by offspring type with results from each simulated population. We chose to average results across simulated populations for our main results because of overall similarity of outcomes. Graphs A and B show the results from analyses that used 18 loci. Graph A uses 95% confidence level cutoff, Graph B uses an 80% confidence level cutoff. Graphs C and D show the results from analyses that used 9 loci. Graph C uses 95% confidence level cutoff, Graph D uses an 80% confidence level cutoff.

**APPENDIX A2: Supplemental Materials for Chapter 2**

**Appendix A2.1.** Site-specific estimates of parasitism using either one line of evidence alone or two combined lines of evidence to identify parasitized nests at 3 sites in Yolo County, CA (Conaway Ranch = CR, n=36; Russell Ranch = RR, n=9; Roosevelt Ranch = Roos, n=59). We assigned nests with insufficient evidence to the Undetermined category. When we used two lines of evidence resulted in one line of evidence determining the nest was parasitized and the other line determining the nest was not parasitized, we assigned the nest to the Conflicting Evidence category.

| **Method** | **Parasitized** | | | | **Not Parasitized** | | | | **Undetermined** | | | | **Conflicting Evidence** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CR | RR | RO | | CR | RR | RO | | CR | RR | RO | | CR | RR | RO |
| **Single Line of Evidence** | | | | | | | | | | | | | | | |
| Genetics | 27 | 6 | 51 | | 8 | 3 | 8 | | 0 | 0 | 0 | | — | — | — |
| RFID | 31 | 6 | 49 | | 3 | 3 | 6 | | 1 | 0 | 4 | | — | — | — |
| Egg Accumulation | 22 | 6 | 27 | | 14 | 3 | 14 | | 0 | 0 | 18 | | — | — | — |
| Clutch Size | | | | | | | | | | | | | | | |
| **10** | 31 | 8 | 56 | | 4 | 1 | 3 | | 0 | 0 | 0 | | — | — | — |
| **12** | 29 | 7 | 52 | | 6 | 2 | 7 | | 0 | 0 | 0 | | — | — | — |
| **14** | 23 | 4 | 45 | | 12 | 5 | 14 | | 0 | 0 | 0 | | — | — | — |
| **16** | 16 | 3 | 32 | | 19 | 6 | 27 | | 0 | 0 | 0 | | — | — | — |
| **18** | 9 | 1 | 26 | | 26 | 8 | 33 | | 0 | 0 | 0 | | — | — | — |
| **20** | 7 | 1 | 21 | | 28 | 8 | 38 | | 0 | 0 | 0 | | — | — | — |
| **22** | 5 | 0 | 15 | | 30 | 9 | 44 | | 0 | 0 | 0 | | — | — | — |
| | | | | | | | | | | | | | | | |
| **Two Lines of Evidence** | | | | | | | | | | | | | | | |
| RFID and EA | 20 | 4 | 23 | | 3 | 1 | 4 | | 0 | 0 | 17 | | 11 | 4 | 11 |
| RFID and Genetics | 25 | 5 | 47 | | 2 | 2 | 3 | | 1 | 0 | 4 | | 7 | 2 | 5 |
| Genetics and EA | 19 | 4 | 25 | | 6 | 1 | 5 | | 0 | 0 | 18 | | 10 | 4 | 11 |

**Appendix A2.2.** Number of parasitic wood duck eggs by method of detection for three nesting sites in Yolo County, CA, a) pooled and b) separated by site. Eggs were classified as undetermined when either 1) we could not determine where an offspring hatched from, as in the case of offspring we encountered for the first time in a post-hatch trapping effort, or 1) RFID was off/shut down for the box the offspring hatched from. Assignments were not supported by RFID only when the RFID was on and the was not scanned during the breeding attempt.

a) Parasitic offspring by method of detection pooled across three field sites in Yolo County, CA (n=1194).

| | Not Parasitic | Parasitic | Undetermined | No RFID Support |
|---|---|---|---|---|
| **Genetics** | 747 | 439 | 8 | — |
| **Genetics Subset** | 729 | 339 | 126 | — |
| **Genetics + RFID** | 668 | 254 | 126 | 146 |

b) Parasitic offspring by method of detection and site. The three sites in this study were Conaway Ranch (CR, n = 417), Russell Ranch (RR, n = 103), and Roosevelt Ranch (RO, n = 669), all located in Yolo County, CA.

| | Not Parasitic | | | Parasitic | | | Undetermined | | | No RFID Support | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CR | RR | RO | CR | RR | RO | CR | RR | RO | CR | RR | RO |
| **Genetics** | 290 | 71 | 385 | 127 | 37 | 276 | 0 | 0 | 8 | — | — | — |
| **Genetics Subset** | 280 | 71 | 378 | 123 | 24 | 192 | 14 | 13 | 99 | — | — | — |
| **Genetics and RFID** | 272 | 71 | 325 | 100 | 24 | 130 | 14 | 13 | 99 | 31 | 0 | 115 |

**Appendix A2.3.** Individual female tactics by method of detection a) across all three field sites and b) separated by field site. Genetics assigned tactics to 110 females, but RFID information was only available for 85 of the females assigned by genetics, so we subset genetically assigned females in order to compare tactics informed by RFID activity to purely genetically assigned tactics.

a) Individual female tactics by method of detection pooled across three field sites in Yolo County, CA (n=110 for Genetics, n = 85 for Genetics Subset/RFID and Genetics).

| Method | Nesting Parasite | Non-Parasite | Parasite Only |
|---|---|---|---|
| **Genetics** | 52 | 36 | 22 |
| **Genetics Subset** | 48 | 32 | 5 |
| **RFID+Genetics** | 34 | 46 | 5 |

b) Individual female tactics by method of detection and site. The invididul female tactics are nesting parasite (NP), non-parasite/nesting only (NO), and parasite only (PO).

| Method | Conaway Ranch | | | Russell Ranch | | | Roosevelt Ranch | | |
|---|---|---|---|---|---|---|---|---|---|
| | NP | NO | PO | NP | NO | PO | NP | NO | PO |
| **Genetics** | 19 | 12 | 5 | 2 | 6 | 3 | 32 | 19 | 14 |
| **Genetics Subset** | 15 | 11 | 3 | 2 | 6 | 1 | 31 | 15 | 1 |
| **RFID and Genetics** | 13 | 13 | 3 | 2 | 6 | 1 | 19 | 27 | 1 |

## APPENDIX A3: Supplemental Materials for Chapter 3

**Appendix A3.1.** Unsampled mothers identified by COLONY by year and site. The three field sites in this study were Conaway Ranch (CR), Roosevelt Ranch (ROOS), and Russell Ranch (RR). No genetic samples were collected from Roosevelt Ranch in 2013. We identified a subset of the females COLONY had identified as unsampled through additional maternity analyses, RFID activity on nests of offspring assigned to the initially unsampled females, and cross checking nest records.

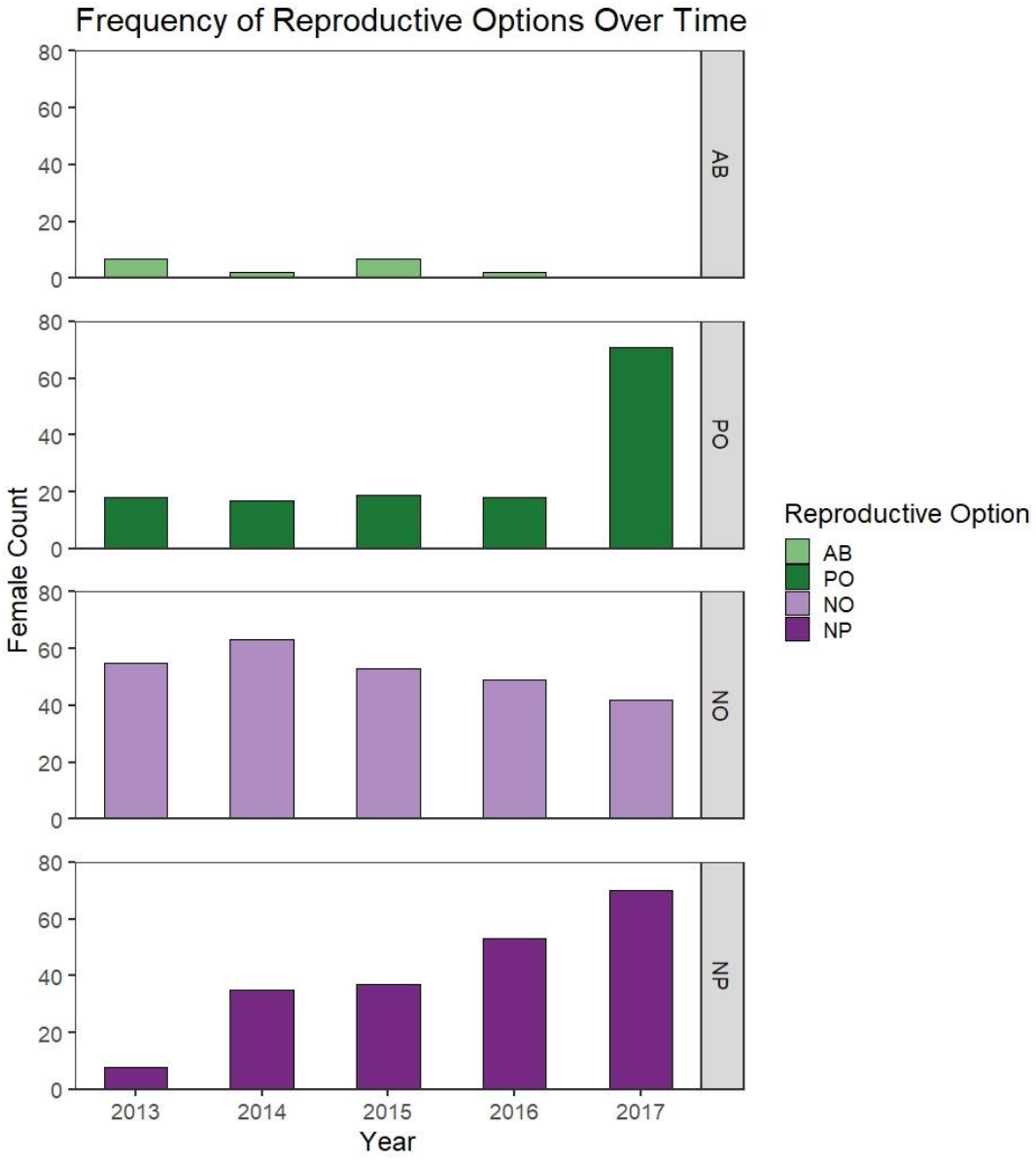
| Year | Site | Initial Unsampled Females | Identified Females | Final Unsampled Females |
|---|---|---|---|---|
| **2017** | CR | 30 | 3 | 27 |
| | ROOS | 20 | 4 | 16 |
| | RR | 9 | 0 | 9 |
| **2016** | CR | 3 | 0 | 3 |
| | ROOS | 12 | 5 | 7 |
| | RR | 2 | 2 | 0 |
| **2015** | CR | 6 | 2 | 4 |
| | ROOS | 13 | 7 | 6 |
| | RR | 1 | 0 | 1 |
| **2014** | CR | 5 | 2 | 3 |
| | ROOS | 16 | 11 | 5 |
| | RR | 0 | 0 | 0 |
| **2013** | CR | 9 | 2 | 7 |
| | ROOS | — | — | — |
| | RR | 3 | 0 | 3 |
| **Total** | | 128 | 38 | 90 |

**Appendix A3.2** . Breeding female densities and nest box densities for each site and year.

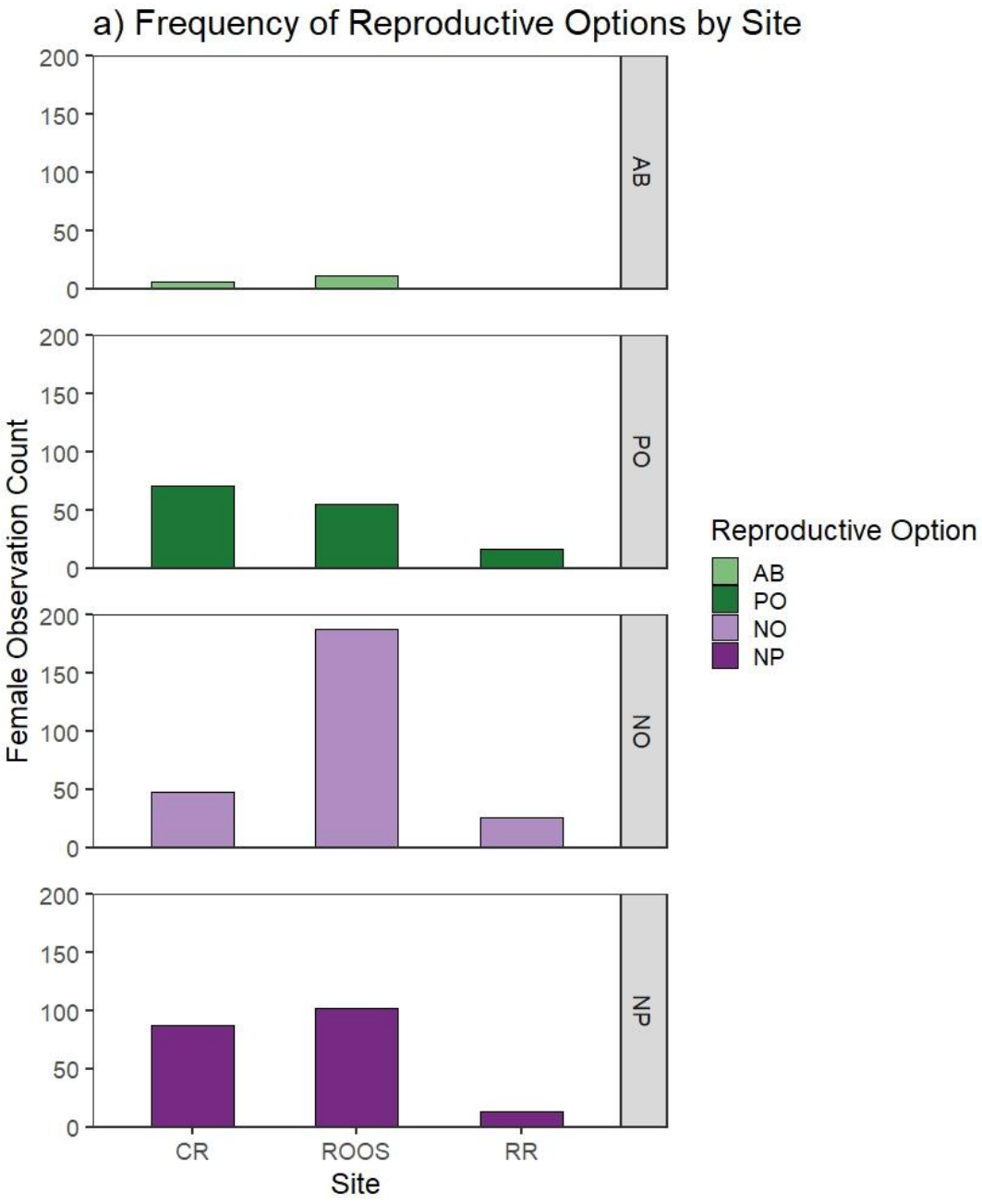
| Site | Year | Breeding Female Density (per Ha) | Nest Box Density (per Ha) |
|---|---|---|---|
| **Russell Ranch** | 2013 | 1.11 | 1.48 |
| | 2014 | 1.48 | 1.61 |
| | 2015 | 1.61 | 1.98 |
| | 2016 | 1.48 | 1.98 |
| | 2017 | 2.35 | 1.98 |
| **Conaway Ranch** | 2013 | 2.03 | 3.58 |
| | 2014 | 2.39 | 3.58 |
| | 2015 | 2.63 | 4.30 |
| | 2016 | 2.51 | 4.30 |
| | 2017 | 4.78 | 4.30 |
| **Roosevelt Ranch** | 2014 | 0.30 | 0.32 |
| | 2015 | 0.34 | 0.32 |
| | 2016 | 0.29 | 0.32 |
| | 2017 | 0.27 | 0.32 |

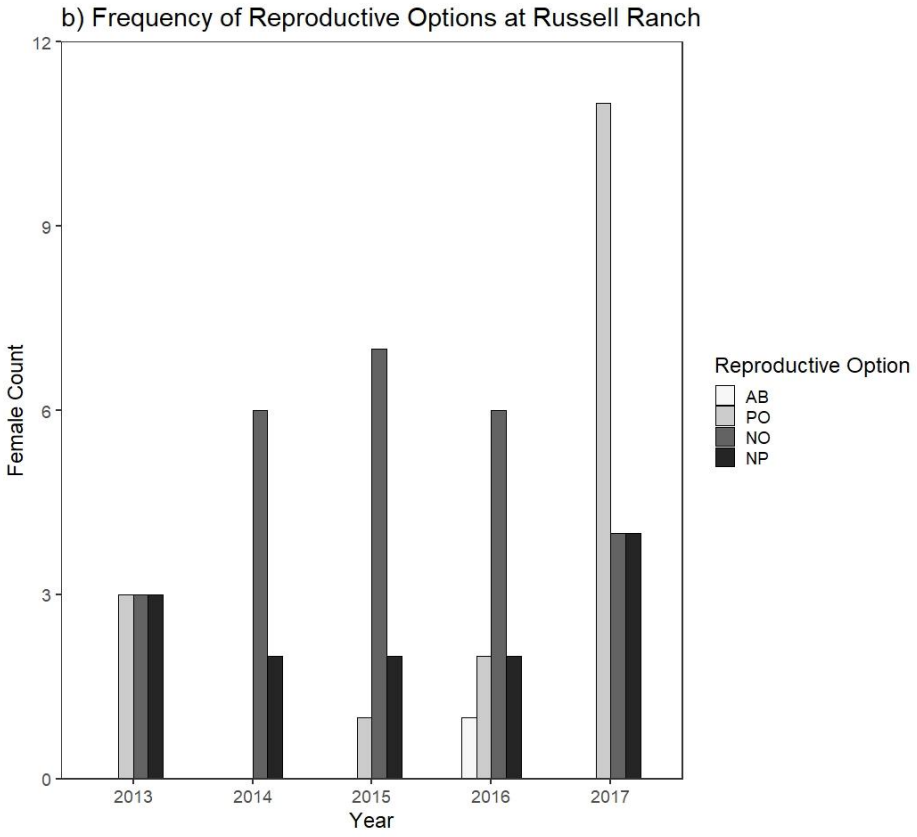
**Appendix A3.3.** Nest fates by site and year. Nests that had at least one offspring hatch successfully were categorized as hatched. Nests that females stopped visiting were categorized as abandoned. Depredated nests had at least one egg missing with signs of predator activity (i.e. broken eggshells, blood). Researchers collected nests when it was apparent that they were either abandoned or otherwise would not hatch.

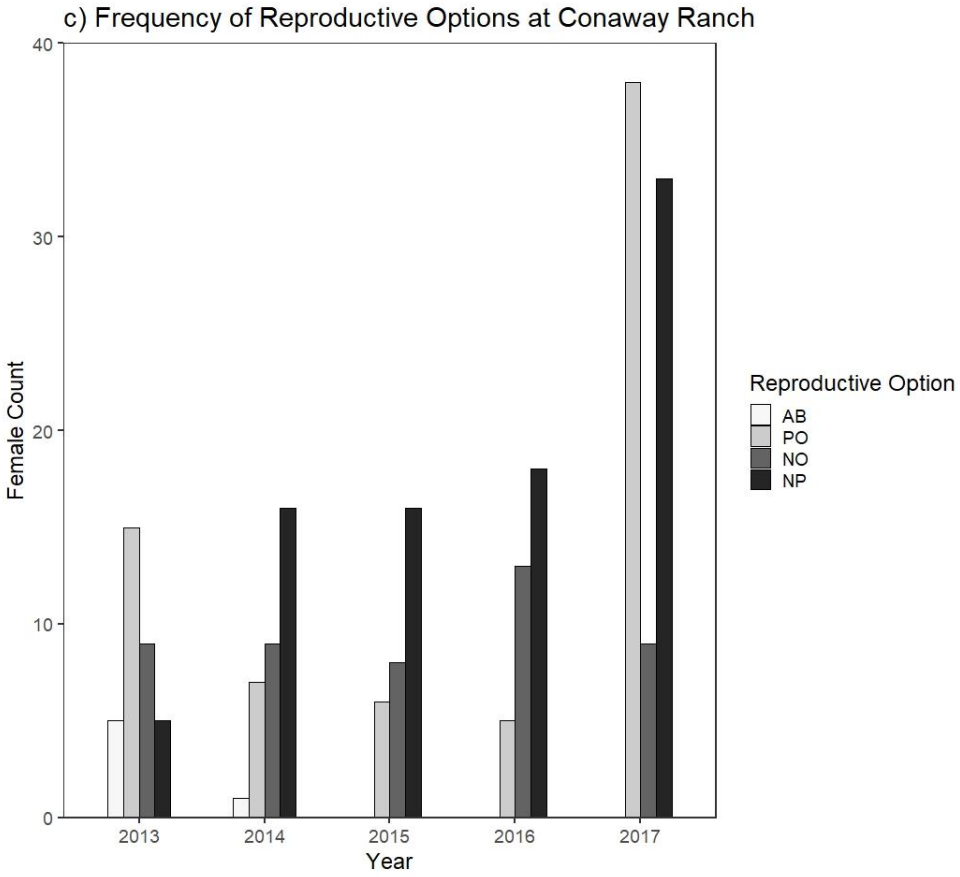
| Site | Year | Hatched | Abandoned | Depredated | Collected | Total |
|---|---|---|---|---|---|---|
| **Russell Ranch** | 2013 | 6 | 1 | 0 | 0 | 7 |
| | 2014 | 8 | 0 | 0 | 0 | 8 |
| | 2015 | 9 | 0 | 0 | 0 | 9 |
| | 2016 | 7 | 1 | 0 | 0 | 8 |
| | 2017 | 9 | 0 | 0 | 0 | 9 |
| **Conaway Ranch** | 2013 | 8 | 5 | 4 | 0 | 17 |
| | 2014 | 20 | 5 | 1 | 0 | 26 |
| | 2015 | 24 | 2 | 2 | 0 | 28 |
| | 2016 | 30 | 1 | 2 | 1 | 34 |
| | 2017 | 39 | 4 | 2 | 0 | 45 |
| **Roosevelt Ranch** | 2014 | 60 | 7 | 1 | 0 | 68 |
| | 2015 | 56 | 4 | 0 | 9 | 69 |
| | 2016 | 52 | 5 | 0 | 12 | 69 |
| | 2017 | 51 | 9 | 0 | 6 | 66 |

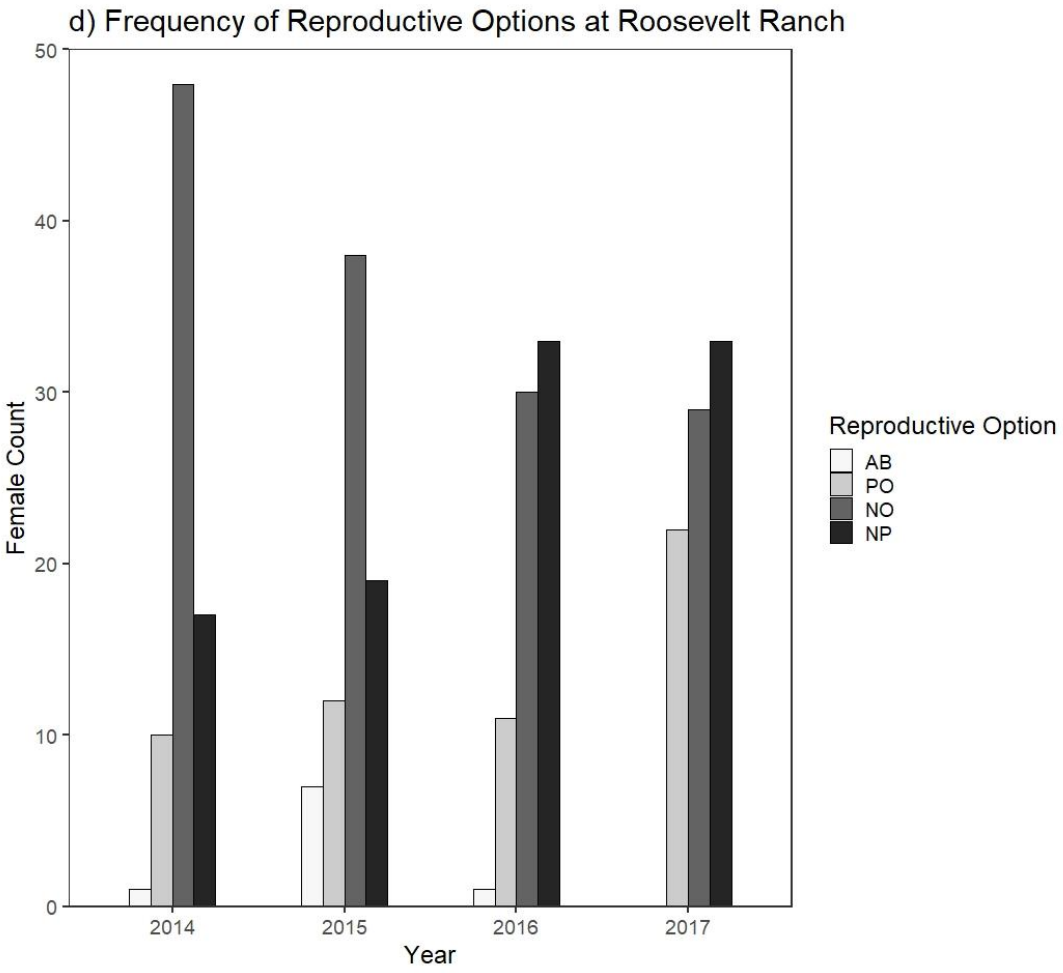



**Appendix A3.4.** The distribution of reproductive options from 2013-2017. Sites were pooled to show the relationship between year and reproductive option. The reproductive options are absent from breeding (AB), parasitize only (PO), nest only (NO), and parasitize and nest (NP). For site-specific distributions of reproductive options across years, see Appendix A3.5.

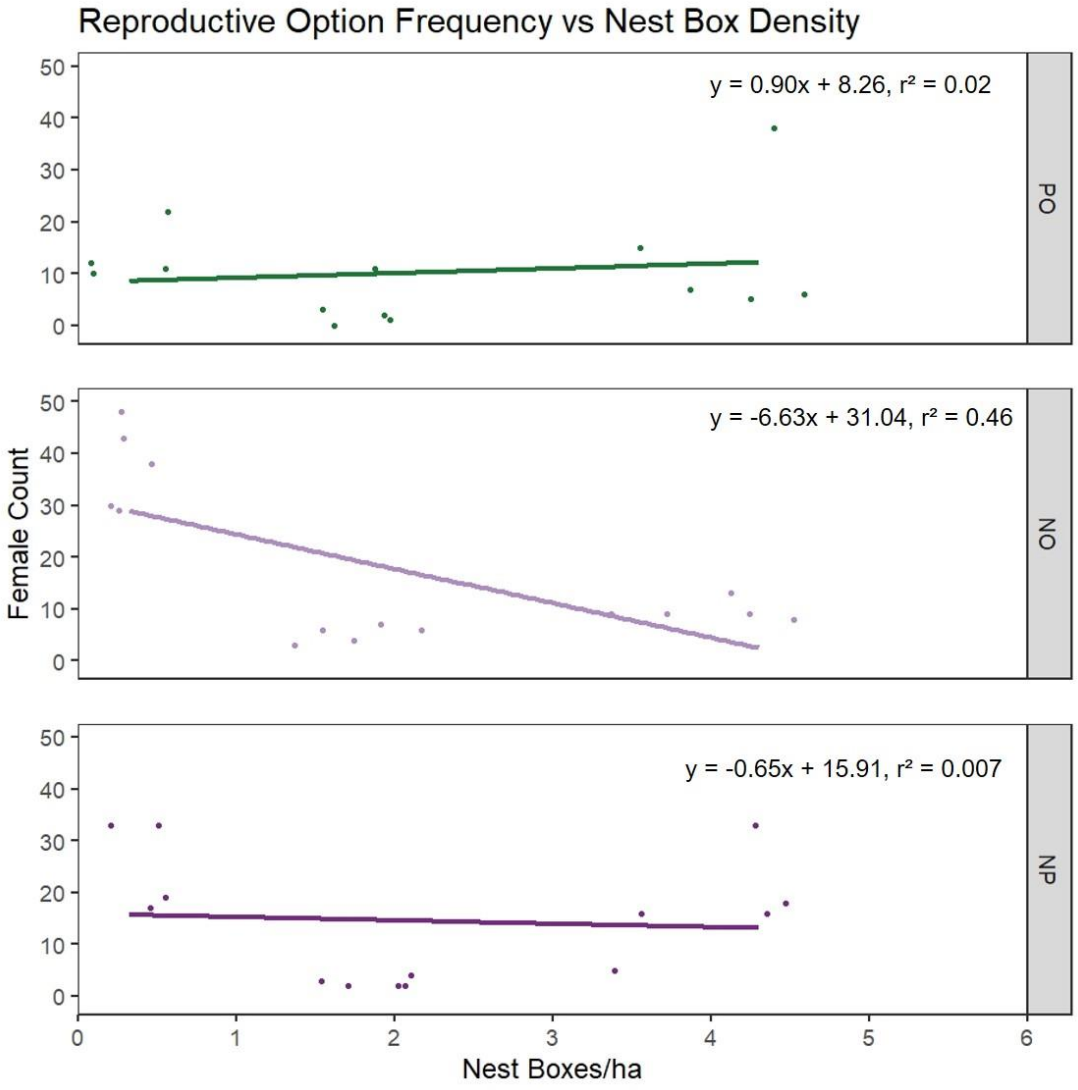

a) Frequency of Reproductive Options by Site
Female Observation Count
200
150
100
50
0
AB
PO
NO
NP
CR
ROOS
RR
Site
Reproductive Option
AB
PO
NO
NP


b) Frequency of Reproductive Options at Russell Ranch
Female Count
12
9
6
3
0
2013
2014
2015
2016
2017
Year
Reproductive Option
AB
PO
NO
NP


c) Frequency of Reproductive Options at Conaway Ranch
Female Count
40
30
20
10
0
2013
2014
2015
2016
2017
Year
Reproductive Option
AB
PO
NO
NP




**Appendix A3.5.** Distribution of reproductive options (as determined by counts of females) from 2013-2017 for (a) all sites, (b) Russell and (c) Conaway ranches, and 2014-2017 for (d) Roosevelt Ranch. The reproductive options are absent from breeding (AB), parasitize only (PO), nest only (NO), and parasitize and nest (NP).




**Appendix A3.6.** The number of females adopting reproductive options in relation to the density of nest boxes per hectare. Nest box density varied slightly between sites and years (see Appendix A3.2) so all sites and years are pooled.